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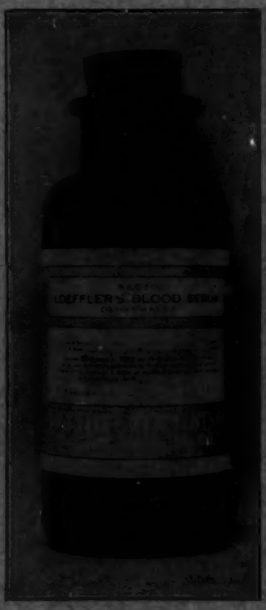

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THE RELATIONSHIP BETWEEN THE HYDROGEN-ION CONCENTRATION AND THE BACTERIAL CONTENT OF COMMERCIAL MILK

EDWIN W. SCHULTZ, ALBERTA MARX, AND HAROLD J. BEAVER

From the Department of Bacteriology and Experimental Pathology, Stanford University, California

During the summer of 1916 the senior author, while working in the department of pediatrics of the Johns Hopkins University, began studies to determine the relationship between the hydrogen ion concentration and the bacterial count of cows' milk. The work then interrupted has recently been continued. The object of this paper is to present the methods employed and the results thus far obtained.

The hydrogen-ion concentration of fresh cows' milk and also that of sour milk, in other words, the range of acidity, has already been determined by a number of workers. Its pH runs from approximately 6.8, for fresh milk, to 4.6, for completely soured milk. On the basis of these facts standard solutions covering this particular range of acidity were prepared.

PREPARATION OF STANDARDS

The standard solutions are prepared by mixing in certain proportions fifteenth molecular solutions each of acid potassium phosphate and alkaline sodium phosphate. These initial solutions are prepared as follows: (a) Fifteenth molecular solution of acid potassium phosphate is prepared by dissolving 9.078 grams of the pure, recrystallized salt (KH_2PO_4), in distilled water and making it up to 1 liter. (b) Fifteenth molecular solution of alkaline sodium phosphate is prepared by dissolving 11.876 grams of the pure, recrystallized, anhydrous, salt ($Na_2HPO_4 \cdot 2H_2O$) in distilled water and making it up to 1 liter. These solutions

are then mixed in the proportions indicated in the following tabulation:

Tabulation of mixtures

	pH							
	7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.3
Acid potassium phosphate, cc	37.00	44.00	50.50	56.67	62.67	68.50	73.00	77.50
Alkaline sodium phosphate, cc	63.00	56.00	49.50	43.33	37.33	31.50	27.00	22.50

	pH							
	6.2	6.1	6.0	5.9	5.8	5.7	5.6	5.5
Acid potassium phosphate, cc	81.25	84.25	87.00	90.00	91.50	93.25	94.75	95.67
Alkaline sodium phosphate, cc	18.75	15.75	13.00	10.00	8.50	6.75	5.25	4.33

	pH		
	5.4	5.3	5.2
Acid potassium phosphate, cc	96.50	97.34	97.75
Alkaline sodium phosphate, cc	3.50	2.66	2.25

From 5.2 to 4.4 Phthalate-NaOH mixtures are employed

	pH				
	5.2	5.0	4.8	4.6	4.4
M/5 KH Phthalate, cc	50.00	50.00	50.00	50.00	50.00
M/5 NaOH, cc	29.95	23.85	17.70	12.15	7.50

Each one of the Phthalate-NaOH mixtures is then diluted to 200 cc. A M/5 KH Phthalate solution contains 40.828 grams of the pure salt in 1 liter. The M/5 should be prepared as free as possible from carbonates.

The standard solutions thus made may be set aside and used as required in the preparation of fresh colorimetric standards to be described presently. All the glass used in the preparation and preservation of the standard solutions should be alkali free, such as Jena, Non-sol, or Pyrex. A crystal or two of thymol should be added to the solutions to prevent the growth of molds.

From the stock solutions a set of standard tubes covering the range from 6.8 to 4.6 is next prepared. A convenient size of tube to use for this purpose is one measuring 10 by 110 mm.

To each tube is added 2 cc. of the standard solution, each tube being carefully labeled with the pH it represents. To the entire series is then added an equal amount of indicator per tube. Two indicators are required to cover the range from 6.8 to 4.6. Brom-thymol blue is used from 6.8 to 6.0 and methyl red from 6.0 to 4.6. Enough of either indicator is added to give the most pronounced color changes in the range for which it is employed, usually one-tenth the volume of the standard solution is sufficient.

PREPARATION OF INDICATOR SOLUTIONS

Brom-thymol blue (dibromthymolsulphonephthalein) is prepared by adding 1 decigram of the powder to 3.2 cc. twentieth normal sodium hydroxide. This is warmed and agitated until the powder goes into solution and sufficient distilled water is added to make 15 cc. This constitutes the stock solution. The test solution is prepared by diluting the stock solution with 30 volumes of distilled water.

Methyl red (orthocarboxybenzeneazodimethylaniline) is prepared by dissolving 1 decigram of the powder in 300 cc. of alcohol and diluting to 500 cc. with distilled water.

PREPARATION OF DIALYSER SACS

To prepare the collodion sacs for dialysing the milk 1 ounce of collodion (Anthony's negative cotton) is dissolved in 500 cc. of a mixture of equal quantities of ether and ethyl alcohol. The solution should stand for several days, at which time the clear supernatent fluid is ready for use. A small test tube (9 by 120 mm.) is filled with the collodion, inverted, and half its contents poured out. The tube is then righted and the collodion allowed to fill the lower half again. It is inverted again and rotated on practically its vertical axis, thereby draining off the excess collodion. The tube is then clamped in an inverted position and allowed to stand until the odor of ether has disappeared. Thereupon it is filled several times with cold water and, after loosening the upper rim with the aid of a knife blade, is removed with gentle traction. The sacs should be preserved by complete immersion in water.

TECHNIC OF THE METHOD

The method of determining the hydrogen-ion concentration of the milk consists of putting about 1 cc. of milk in the sac and lowering it into a test tube of the same diameter as those used for the standards and containing 2 cc. of normal (0.8 per cent) salt solution of a neutral reaction. It requires five minutes for dialysis to take place. The sac is then removed, the same amount of indicator used in the standards is added and thoroughly mixed. The reading is made by comparing the tube with the series of standard tubes until the corresponding color is found. This is done with the aid of a simple comparator having a white glass background and in the presence of good light.

BACTERIAL COUNTS

The bacterial counts were made in accordance with the standard methods set forth by the American Public Health Association in 1916. The milk was plated in each instance immediately after the pH readings were made. The milk used was from a city dairy receiving milk from a number of sources.

RESULTS

The results thus far obtained are given on the following chart. The curve represents the averages of the bacterial counts obtained for each pH from 6.8 to 4.6. More determinations would make it, when considered together with the variations in the counts at a given pH, of greater interest and significance.

DISCUSSION

The results thus far obtained do not permit the drawing of definite conclusions. It is suggestive, however, that at a pH of 6.6 the count begins to run high, averaging 16,000,000; at 6.5 reaching an average of 100,000,000, etc., though at these pH readings the acidity cannot be detected by taste. The acidity is first detectable by taste at about a pH of 6.0.

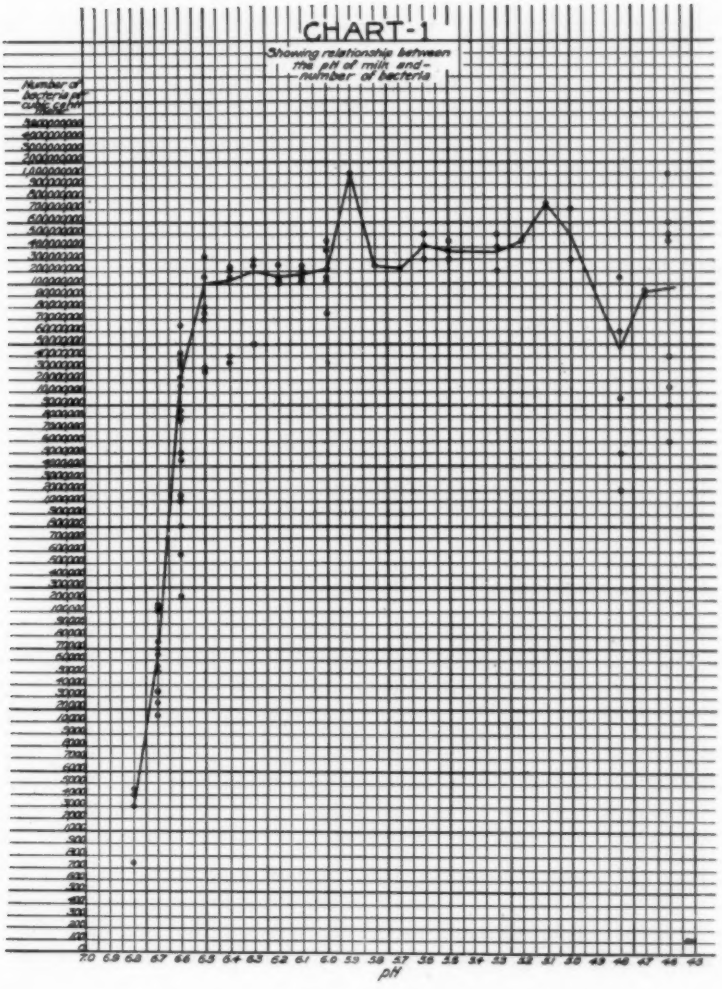
CHART-1
Showing relationship between the pH of milk and number of bacteria
Number of bacteria per cubic cm/ml
7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5
pH

From these observations it seems possible that with further determinations a curve of average counts for the range of acidity will be obtained which shall be of value in estimating the number of bacteria in milk, a matter of a few minutes rather than hours required for actually making bacterial counts. This method would therefore be of value in children's institutions and other places where it is of importance to determine immediately the quality of milk before its consumption.

GRADING MILK BY THE ACID TEST: INFLUENCE OF ACIDS IN THE RATION ON THE ACIDITY OF MILK[1]

H. H. SOMMER AND E. B. HART

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In grading milk by means of the acid test, milk with an acidity of over 0.18 per cent is usually rejected. This practice has revealed a number of herds where the acidity of the fresh milk is greater than 0.18 per cent (1), (2), (3). In some of these cases attempts have been made to lower the acidity by a change in the ration; farmers have been advised to quit feeding silage or bran. This raises two questions:

First: Is milk with a high apparent acidity (over 0.18 per cent) undesirable?

Second: Is the acidity of the milk influenced by acids, organic or inorganic, in the ration?

IS MILK WITH A HIGH APPARENT ACIDITY UNDESIRABLE?

The practice of rejecting milk with a high acidity is used most commonly by condenseries to guard themselves against losses as a result of coagulation on sterilizing the evaporated milk. It is undoubtedly true that milk that has a high acidity due to fermentation will not withstand sterilization; and it is only just that such milk should be rejected. However it does not follow that fresh milk with a high apparent acidity is also undesirable. On the contrary it has been shown by Sommer and Hart that there is no relation between apparent acidity and the heat coagulation under pressure at 136°C. (4).

The following table is reproduced to illustrate this fact.

Out of the 86 samples of fresh milk ranging in acidity from 0.102 to 0.257 per cent, 45 had an acidity of over 0.18 per cent,

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

and 41 below 0.18 per cent. Of the 45 samples above 0.18 per cent in acidity, 23 coagulated within twenty minutes when heated under pressure to 136°C.; and of the 41 samples below 0.18 per cent 19 coagulated. This indicates that there is no relation between apparent acidity and coagulation.

The apparent acidity, due to the acid salts and casein, is not an index to the hydrogen ion concentration in the milk. A high apparent acidity does not indicate a high hydrogen ion concentration, and does not in any case cause a sour taste or odor. A sample of milk from the University herd with an acidity of 0.257 per cent did not taste or smell sour. Knowing what the apparent acidity is due to, we do not expect such a sample of milk to taste sour.

TABLE 1

Summary of titratable acidity and coagulation

DATE	TOTAL SAMPLES	ACIDITY ABOVE 0.18 PER CENT		ACIDITY BELOW 0.18 PER CENT	
		Number of samples	Number coagulating in 20 minutes	Number of samples	Number coagulating in 20 minutes
May 8.....	26	15	5	11	6
May 10.....	30	14	7	16	7
May 16.....	30	16	11	14	6
Total.....	86	45	23	41	19

We can conclude that milk with a high apparent acidity is not undesirable. There is no justification for rejecting such milk if normal in other respects; and there is no object in attempting to lower the acidity by a change in the ration.

CAN THE ACIDITY OF THE MILK BE INFLUENCED BY ACIDS IN THE RATION?

The question of whether or not the organic acids of silage increase the acidity has been studied, and the results showed clearly that there is no influence (5). However, the inorganic acids, that can not be eliminated from the body by oxidation, might cause a rise in the acidity of the milk. To offer experimental data on this point the following experiment was conducted.

EXPERIMENTAL

Sulphuric acid was added to the ration of a normal healthy cow of the University herd. The titratable acidity, hydrogen ion concentration, and coagulation of the milk, and the reaction of, and distribution of nitrogen in the urine were carefully studied.

The original ration consisted of 30 pounds of silage, mixed hay, and 12 pounds of grain mixture. In feeding the acid, the 30 pounds silage was replaced by a mixture consisting of 15 pounds silage, 6 pounds dry corn stover, and 9 pounds water plus the sulphuric acid. The following table gives the results.

From the above data it is seen that even 120 cc. of concentrated sulphuric acid given daily did not produce a change in the acidity of the milk. The hydrogen ion concentration of the milk also remained constant, although the urine became distinctly acid. The urine was distinctly alkaline before the acid was fed, having a pH of 8.21 and only 0.27 per cent of the nitrogen in the form of ammonium salts. During the experiment the urine became distinctly acid having a pH of 5.83 on January 19, and 6.35 per cent of the nitrogen in the form of ammonium salts on January 16. This shows that the acid was absorbed into the blood stream and that it required a distinct effort to eliminate it. From these data we can conclude that the ration does not influence the acidity of the milk.

A SUGGESTED MODIFICATION IN THE USE OF THE ACID TEST

We are confronted with these two facts: (1) the high apparent acidity of milk is not undesirable, and (2) even if it were, it could not be reduced by a change in the ration. We should, therefore, use a test in grading milk which will not reject normal fresh milk with a high apparent acidity.

The ideal test for grading milk should be a measure of the extent to which the normal milk has undergone change. The acid test as it is now used is not such a measure, it merely measures acidity without differentiating between apparent acidity and acidity due to fermentation. However, it could be modified to approach the ideal test.

TABLE 2

Data on reaction of milk and urine

DATE	MILK			URINE				
	Titratable acidity	pH	Coagulation	Time voided	pH	Per cent total N	Per cent ammoniacal N	Per cent of total N as NH_3
			minutes					
December 12	0.132	6.76	20–					
December 19	0.132	6.745	20–					
December 20	0.128	6.75	20–					
December 23	0.132	6.75						
December 24	0.139	6.78	20–					
December 26	0.124	6.765	20–					
December 27	0.124	6.76	20–					
December 28	0.128	6.75						
December 29	0.128	6.76	20–	4.30 a.m.	8.21	1.61	0.0044	0.27
Changed from original ration, giving 30 cc. of concentrated sulphuric acid daily								
December 30	0.128	6.77						
December 31	0.128	6.78						
January 2	0.139	6.75	20–					
January 3	0.132	6.78	20–	2.15 p.m.	7.30	1.84	0.0086	0.47
January 4	0.128	6.82						
Increased to 40 cc. of concentrated sulphuric acid								
January 5	0.135	6.77						
January 6	0.132	6.75						
January 7	0.130	6.77		4.30 a.m.	7.98	1.25	0.0036	0.28
January 8	0.132	6.77		2.30 p.m.	7.71	0.64	0.0020	0.31
Increased to 80 cc. of concentrated sulphuric acid								
January 9	0.132	6.75						
January 10	0.132	6.76		2.00 p.m.	6.897	1.03	0.004	0.39
January 10	0.132	6.74						
January 11	0.132	6.76						
January 12	0.135	6.73		2.15 p.m.	6.072	0.946	0.0244	2.5
January 13	0.132	6.725	20–					
January 14	0.135	6.72						
Increased to 120 cc. concentrated sulphuric acid								
January 15	0.135	6.70						
January 16	0.132	6.70	20–	4.00 p.m.	6.102	0.672	0.0424	6.35
January 17	0.132	6.70						
January 19	0.128	6.76		4.30 a.m.	5.83	1.331	0.0468	3.50
January 20	0.128	6.73	20–	4.30 p.m.	5.94	0.93	0.0296	3.18
January 21	0.128	6.78						
Changed back to original ration								
January 22	0.135	6.73						
January 24	0.128	6.76	20–					
January 26	0.117	6.78						

The following procedure is suggested, especially in cases where the acidity is persistently high and very evidently due to a high apparent acidity.

1. Determine the apparent acidity of the milk at intervals of several weeks. The acidity runs quite constant.

2. Allow an increase in acidity (e.g., 0.03 per cent) due to slight fermentation that is likely to occur before delivery.

3. If the apparent acidity at the farm was 0.17 per cent then reject the milk if the acidity exceeds 0.20 (0.17 + 0.03).

SUMMARY AND CONCLUSIONS

1. Milk with a high apparent acidity is not undesirable.

2. The acidity of the milk is not influenced by the ration.

3. In cases where the high acidity of the milk delivered is due to a high apparent acidity and not to fermentation, the acid test should be so modified that it will accept such milk.

REFERENCES

(1) McINERNEY, T. J.: Jour. of Dairy Sc., 1920, iii, no. 3.
(2) SOMMER, H. H., AND HART, E. B.: Jour. of Bio. Chem., 1919, xl, no. 1.
(3) From direct and indirect reports to us.
(4) SOMMER, H. H., AND HART, E. B.: Jour. of Bio. Chem., 1919, xl, no. 1.
(5) TURNER, B. B., AND BEACH, C. L.: Storr's Ag. Exp. Sta., Report 1904.

THE VALUE OF THE PUREBRED SIRE IN INCREASING THE PRODUCTION OF A SCRUB HERD[1]

ANDREW C. McCANDLISH

Dairy Husbandry Section, Agricultural Experiment Station, Ames, Iowa

Preliminary reports from the Iowa Station have shown the profound influence of environment and breeding in increasing the production of a scrub herd and the results which have become available since the publication of these reports further emphasize the purebred dairy sire as a valuable factor in increasing the producing ability of a herd.

PLAN OF INVESTIGATION

In the summer of 1907 a number of scrub cows were purchased in Arkansas and moved to the Iowa Station. These animals were very inferior individuals and had received very poor feed and care. They were of no known breeding, were very unprepossessing in appearance and gave no indications of being valuable dairy animals. The influence of good feed and care on the production of these animals was studied, as has already been reported and at the same time they were used in the breeding investigation.

The scrub cows were mated to purebred dairy bulls of the Holstein, Guernsey and Jersey breeds. The heifers resulting from such matings were saved and mated to bulls of the same breed as their sires. Milk and butterfat records have now been obtained on two generations of grades and some of the third generation, or those carrying 87½ per cent of the blood of

[1] This is supplementary to work already published, viz.:

Influence of Environment and Breeding in Increasing Dairy Production. 1916. H. H. Kildee and A. C. McCandlish, Ia. Ag. Exp. Sta. Bul. 165.

Influence of Environment and Breeding in Increasing Dairy Production. II. A. C. McCandlish, L. S. Gillette and H. H. Kildee, Ia. Ag. Exp. Sta. Bul. 188, 1919.

the recognized dairy breeds, are now in the herd, though their production records are not as yet available.

The records used in comparing the scrub, half-blood, and three-quarter-blood cows are all on the mature basis. If a heifer has only one record, it is calculated to the mature basis; where more records are available, then they are placed on the mature basis and the average of the records secured. For computing the record on the mature basis, the following percentages secured at this station from a study of the records of 10,000 cows were used.

TABLE 1

Percentage of mature production expected of immature heifers

AGE	PER CENT
Yearlings	70
Two-year-olds	80
Three-year-olds	85
Four-year-olds	95

The factors studied in this investigation are of a very varied character but only those relating to the use of purebred dairy sires need be mentioned here.

DISCUSSION OF RESULTS

A number of grade animals sired by purebred bulls and descended from the scrub cows have now completed records. These will be studied in two groups—the first generation grades, or those carrying 50 per cent of the blood of one of the recognized dairy breeds, and the second generation grades, or those carrying 75 per cent of the blood of one of those breeds. The only way to determine correctly the value of a bull is to compare the records of his daughters with those of their dams, though there are difficulties connected even with this method as will be shown later. This method can not be used for the purposes of comparing breeds as all bulls were not mated with the same cows and so were not given equal opportunities to demonstrate their abilities as sires of producers.

FIRST GENERATION GRADES

All the first generation of grades sired by a purebred Holstein bull showed an increase over their dams in milk and butterfat production. The increase varied from 38 per cent in fat and 79 per cent in milk to 68 per cent in fat and 121 per cent in milk, while on the average it was an increase of 89 per cent in milk and 58 per cent in fat.

TABLE 2

First generation grades compared with their scrub dams

DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Milk	Fat
Holsteins									
		pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
8	3	2339.5	124.35	68	3	5180.0	209.61	121	68
52	7	3742.3	169.16	69	5	6700.5	282.11	79	67
56	3	3874.6	192.62	77	6	6955.5	266.25	79	38
60	6	3313.2	178.47	207	4	6306.2	287.76	90	61
Guernseys									
6	8	3715.3	164.93	110	3	3821.1	163.05	3	−1
31	7	3463.3	168.00	288	2	5400.8	308.99	56	84
33	3	4338.5	183.49	87	4	4213.1	179.72	−3	−2
52	7	3742.3	169.16	308	2	5355.9	280.78	43	66
53	7	5258.9	233.63	180	3	3639.0	180.53	−31	−23
				253	3	6128.4	298.33	17	28
58	3	3034.5	152.54	175	3	6286.1	322.71	107	112
Jerseys									
31	7	3463.3	168.00	174	3	5009.0	263.72	45	57
53	7	5258.9	233.63	213	4	4274.5	225.74	−19	−3
60	6	3313.2	178.47	241	2	6137.9	349.42	85	96

In the case of the first generation of Guernsey grades, an even wider variation was noticed. It varied from a decrease of 31 per cent in milk and 23 per cent in fat, due to the use of a poor bull, to an increase of 107 per cent in milk and 112 per cent in fat yield. The Guernsey first grade group contained animals that showed the greatest increase and also the greatest decrease

in fat production from their dams. The average increase in yield was 17 per cent in milk and 27 per cent in butterfat.

The first grade Jerseys showed changes in production as compared with their dams that varied from a decrease of 19 per cent in milk and 3 per cent in fat to an increase of 85 per cent in milk and 96 per cent in butterfat. The average increase in their case was 22 per cent in milk and 34 per cent in fat production.

TABLE 3

Averages for first generation grades and their scrub dams

GROUP	DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
	Number of cows	Number of lactations	Milk	Fat	Number of cows	Number of lactations	Milk	Fat	Milk	Fat
			pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
Holstein....	4	19	3406.2	168.74	4	18	6444.4	265.92	89	58
Guernsey...	6	35	4186.0	189.39	7	20	4899.8	240.96	17	27
Jersey.....	3	20	4046.7	194.11	3	9	4933.4	265.88	22	34
Average...	9	47	3968.6	185.66	14	47	5497.8	255.32	39	37

All of the first generation grades when taken as a group showed an increase of 39 per cent in milk and 37 per cent in fat production as compared with their dams.

SECOND GENERATION GRADES

The grades of the second generation ranked high in production, yielding on the average 375.81 pounds of fat per year as compared with a production of 261.93 pounds by their first grade dams and 182.40 pounds by their scrub grand-dams. In every case the production of the second generation grades was at least 50 per cent greater than that of their scrub grand-dams.

The average increase in production for the second generation Holstein grades as compared to their scrub grand-dams was 174 per cent in milk and 130 per cent in fat; the increase was 72 per cent in milk and 94 per cent in fat in the case of the Guernsey grades; and for the Jersey second grades it was 59 per cent in milk and 64 per cent in fat production.

TABLE 4

Two generations of grades compared with their scrub ancestors

DAMS				DAUGHTERS				GRAND-DAUGHTERS				INCREASE IN PRODUCTION			
												First generation		Second generation	
Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Milk	Fat	Milk	Fat
Holsteins															
		pounds	*pounds*			*pounds*	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*	*per cent*	*per cent*
52	7	3742.3	169.16	69	5	6700.5	282.11	281	2	9409.2	347.41	79	67	151	105
								323	1	8847.3	352.69			136	108
56	3	3874.6	192.62	77	6	6955.5	266.25	233	3	13366.2	497.90	79	38	245	158
								282	1	10629.4	402.03			174	109
60	6	3313.2	178.47	207	4	6306.2	287.76	311	2	11069.4	472.31	90	61	234	165
Guernseys															
33	3	4338.5	183.49	87	4	4213.1	179.72	236	2	6345.7	320.16	−3	−2	46	74
								296	2	10283.0	491.74			137	168
53	7	5258.9	233.63	180	3	3639.0	180.53	301	1	8270.1	427.41	−31	−23	57	83
58	3	3034.5	152.54	175	3	6286.1	322.71	298	2	5955.0	309.40	107	112	96	103
								343	1	8521.8	435.85			181	185
Jerseys															
31	7	3463.3	168.00	174	3	5009.0	263.72	245	1	5411.4	287.14	45	57	56	71
60	6	3313.2	178.47	241	2	6137.9	349.42	348	1	5366.9	278.70	85	96	62	56

TABLE 5

Averages for two generations of grades and their scrub ancestors

GROUP	DAMS				DAUGHTERS				GRAND-DAUGHTERS				INCREASE IN PRODUCTION			
													First generation		Second generation	
	Number of cows	Number of lactations	Milk	Fat	Number of cows	Number of lactations	Milk	Fat	Number of cows	Number of lactations	Milk	Fat	Milk	Fat	Milk	Fat
			pounds	*pounds*			*pounds*	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*	*per cent*	*per cent*
Holstein.....	3	16	3673.8	167.36	3	15	6757.5	275.66	5	9	10063.2	385.46	84	65	174	130
Guernsey.....	3	13	4496.6	199.62	3	10	4843.8	229.74	5	8	7744.9	388.23	8	15	72	94
Jersey.....	2	13	3394.0	172.52	2	5	5460.5	298.00	2	2	5389.2	282.92	61	73	59	64
Average.....	7	36	3847.0	182.40	8	30	5944.7	261.93	12	19	8311.4	375.81	55	44	116	106

The average increase in production for the second generation of grades, when all breeds are combined, was 116 per cent in milk and 106 per cent in fat production—a real tribute to the value of the purebred dairy sire.

POTENCY OF SIRES USED

The importance of the purebred dairy sires has been demonstrated by the work under consideration but another fact of value has also been brought out.

A comparison of the two Guernsey bulls that sired the first generation of Guernsey grades shows that there is a great difference in the value of bulls so far as their ability to sire good producing heifers is concerned.

TABLE 6

Daughters of "Fullwood Hopeful" compared with their scrub dams

DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Milk	Fat
		pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
6	8	3715.3	164.93	110	3	3821.1	163.05	3	−1
33	3	4338.5	183.49	87	4	4213.1	179.72	−3	−2

The purebred Guernsey bull "Fullwood Hopeful" had two daughters which averaged only 4045.1 pounds of milk and 172.58 pounds of butterfat while their dams produced nearly as much or 3885.2 pounds of milk and 169.99 pounds of fat.

TABLE 7

Daughters of Imp. Rouge II's Son compared with their scrub dams

DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Milk	Fat
		pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
31	7	3463.3	168.00	288	2	5400.8	308.99	56	84
52	7	3742.3	169.16	308	2	5355.9	280.78	43	66
53	7	5258.9	233.63	180	3	3639.0	180.53	−31	−23
				253	3	6128.4	298.33	17	28
58	3	3034.5	152.54	175	3	6286.1	322.71	107	112

On the other hand, Imp. Rouge II's Son demonstrated his ability to sire good producing cows, his five daughters averaging 5360.1 pounds of milk and 275.71 pounds of fat while their dams produced only 4295.6 pounds of milk and 194.05 pounds of fat.

TABLE 8

A comparison of two Guernsey bulls—Imp. Rouge II's Son and "Fullwood Hopeful"

BULL	DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
	Number of cows	Number of lactations	Milk	Fat	Number of cows	Number of lactations	Milk	Fat	Milk	Fat
			pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
"Fullwood Hopeful"	2	11	3885.2	169.99	2	7	4045.1	172.58	4	2
Imp. Rouge II's Son	4	24	4295.6	194.05	5	13	5360.1	275.71	25	43

The bull "Fullwood Hopeful" sired daughters which produced only 2 per cent more butterfat than did their scrub dams and consequently can be looked on as having done nothing to improve the production of the herd. On the other hand Imp. Rouge II's Son was of considerable value as his daughters produced 43 per cent more butterfat than did their scrub dams. This instance shows that even a purebred dairy sire should be carefully chosen if the results are to be entirely satisfactory.

TABLE 9

Three-quarter-blood daughters of Imp. Rouge II's Son compared with their dams

DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Milk	Fat
		pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
87	4	4213.1	179.72	236	2	6345.7	320.16	51	78
				296	2	10283.0	491.74	144	174

In studying the second generation of Guernsey grades interesting information is also evident regarding the value of the bulls used. The bulls Imp. Rouge II's Son, and Rouge II's

Holden were half brothers, both being out of the cow Imp. Rouge II of the Brickfield, and Rouge of Ames was a son of Imp. Rouge II's Son. The close relationship of these bulls renders this study interesting.

TABLE 10

Three-quarter-blood daughters of Rouge II's Holden compared with their dams

DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Milk	Fat
		pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
175	3	6286.1	322.71	343	1	8521.8	435.85	36	35

Imp. Rouge II's Son had two daughters out of a daughter of "Fullwood Hopeful" and they were both much better producers than their dam. Their average production was 405.95 pounds of butterfat or 126 per cent more than their dams. Rouge II's Holden had one daughter, out of a daughter of Imp. Rouge II's Son, and her average production was 435.85 pounds of butterfat or 35 per cent more than her dam. There is too little evidence here on which to rank the bulls absolutely according to their ability to sire producers, especially as they were mated to animals of different breeding and producing ability but it is interesting to note that their daughters had about the same average production.

TABLE 11

Three-quarter-blood daughters of Rouge of Ames compared with their dams

DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Milk	Fat
		pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
175	3	6286.1	322.71	298	2	5955.0	309.40	−5	−4
180	3	3639.0	180.53	301	1	8270.1	427.41	127	137

Rouge of Ames was mated to two half-blood daughters of Imp-Rouge II's Son and the heifers resulting were both of good producing ability, one giving an average of 309.40 pounds of fat or

4 per cent less than her dam and the other 427.41 pounds of fat or 137 per cent more than her dam. Though there are these discrepancies in the increase obtained through the use of this bull it is evident that he was of considerable value as his daughters produced on the average 348.74 pounds of fat or 39 per cent more than their dams.

TABLE 12

Three Guernsey bulls compared on basis of three-quarter-blood daughters

BULL	DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
	Number of cows	Number of lactations	Milk	Fat	Number of cows	Number of lactations	Milk	Fat	Milk	Fat
			pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
Imp. Rouge II's Son...	1	4	4213.1	179.72	2	4	8314.3	405.95	97	126
Rouge II's Holden.....	1	3	6286.1	322.71	1	1	8521.8	435.85	36	35
Rouge of Ames.....	2	6	4963.8	251.62	2	3	6726.7	348.74	36	39

It is apparent from the results obtained through the use of these three bulls of similar breeding on the first generation of Guernsey grades that they were all of considerable value in increasing the production of the herd. These daughters had records which were quite comparable and although they did not all bring about the same percentage increase in production it must be remembered that they were mated to cows of very dissimilar producing powers.

INDIVIDUALITY AS A FACTOR IN PRODUCTION

It is a recognized fact that animals of the same breeding may vary widely in producing ability and several good illustrations of this are to be found in the records presented.

If the half-blood Guernsey daughters of Imp. Rouge II's Son out of the scrub cow 53 be studied it will be found that cow 180 produced on the average 31 per cent less milk and 23 per cent less fat than did her dam while cow 253 produced 17 per cent more milk and 28 per cent more fat than did her dam.

Comparable results are to be found in the case of the cows 236 and 296 which are three-quarter-blood Guernseys by Imp. Rouge II's Son and out of the half-blood Guernsey no. 87. These heifers were both better producers than their dam but the increase in butterfat production was 74 per cent in the case of cow 236 and 168 per cent in the case of cow 296.

The two cows 281 and 323 were also full sisters being by the Holstein bull Spring Farm King Pontiac 8th and out of the

TABLE 13

Studies in individuality

DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
Cow number	Number of lactations	Milk	Fat	Cow number	Number of lactations	Milk	Fat	Milk	Fat
Half-blood daughters of Guernsey bull—Imp. Rouge II's Son									
		pounds	*pounds*			*pounds*	*pounds*	*per cent*	*per cent*
53	7	5258.9	233.69	180	3	3639.0	180.53	−31	−23
				253	3	6128.4	298.33	17	28
Three-quarter-blood daughters of Guernsey bull—Imp. Rouge II's Son									
87	4	4213.1	179.72	236	2	6345.7	320.16	46	74
				296	2	10283.0	491.74	137	168
Three-quarter-blood daughters of Holstein bull—Spring Farm King Pontiac 8th									
69	5	6700.5	282.11	281	2	9409.2	347.41	151	105
				323	1	8847.3	352.69	136	108

half-blood Holstein cow 69. They were very similar in producing ability showing almost equal increases in production of butterfat when compared with their dam.

This indicates that a knowledge of the breeding of an animal is not an absolute criterion on which to base an opinion of its producing ability, as the factor of individuality enters in and though breeding is one of the most important factors in determining the producing ability of a herd yet each animal must be judged on its own merits.

SUMMARY

In the work that has been done at the Iowa Station on the grading up of a scrub herd through breeding it has been found that the use of purebred sires is a sure way of increasing milk and butterfat production. It has also been demonstrated, however, that if the best results are to be obtained, the bulls to be used must be selected carefully, as some purebred bulls are not fit to head a scrub herd. The importance of individual variations of necessity demand attention and, even where there is in use a bull that is known to sire good producing heifers, his daughters must be tested as some of them may not come up to the standard of perfection set for the herd. When all the facts of the case are considered the use of good purebred dairy sires is absolutely vindicated.

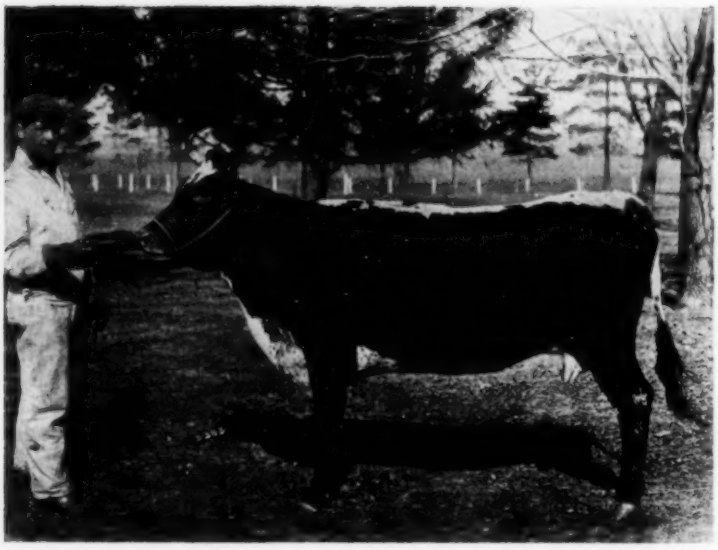

FIG. 1. SCRUB COW NO. 56
Average production 3874.6 pounds of milk and 192.62 pounds of fat

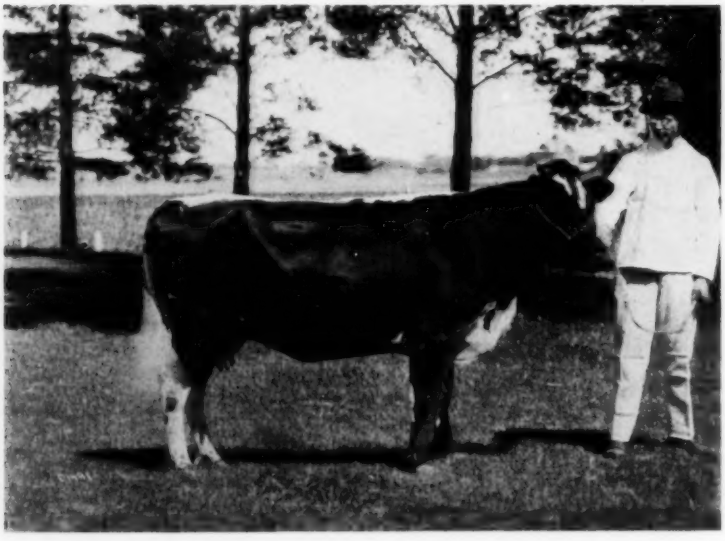

FIG. 2. HALF-BLOOD HOLSTEIN NO. 77, OUT OF SCRUB NO. 56
Average production 6955.5 pounds of milk and 266.25 pounds of fat

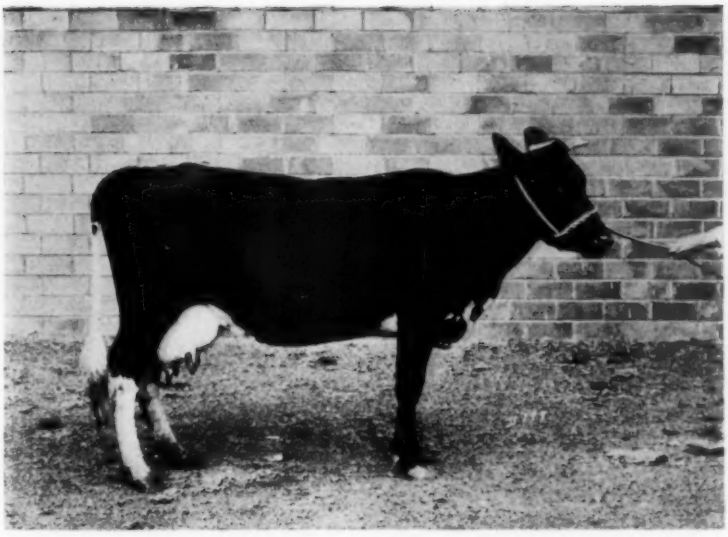

FIG. 3. THREE-QUARTER-BLOOD HOLSTEIN NO. 233, OUT OF HALF-BLOOD HOLSTEIN NO. 77

Average production 13,366.2 pounds of milk and 497.90 pounds of fat

FIG. 4. SCRUB COW NO. 33

Average production 4338.5 pounds of milk and 183.49 pounds of fat

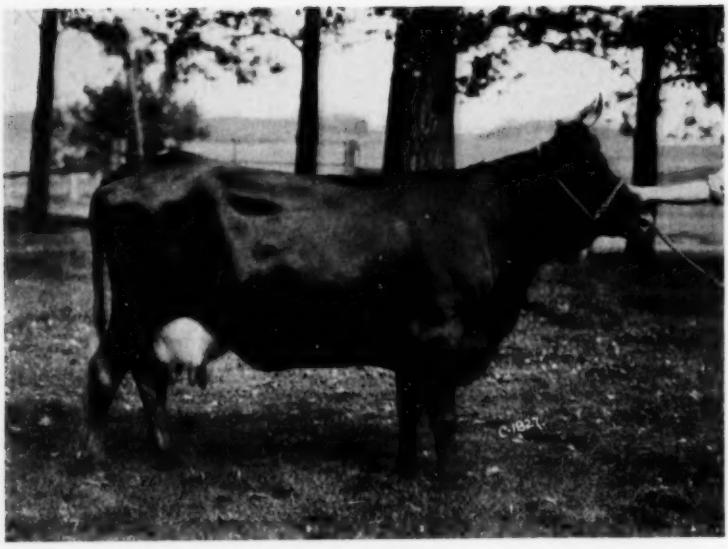

FIG. 5. HALF-BLOOD GUERNSEY NO. 87, OUT OF SCRUB NO. 33
Average production 4213.1 pounds of milk and 179.72 pounds of fat

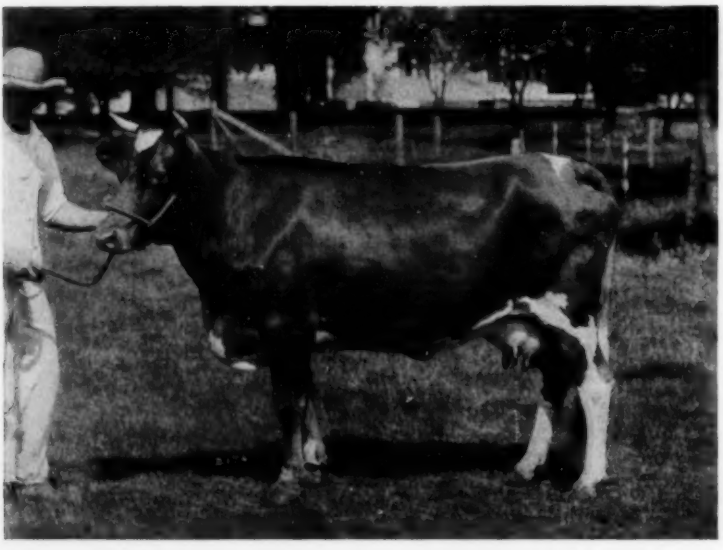

FIG. 6. THREE-QUARTER-BLOOD GUERNSEY NO. 236, OUT OF HALF-BLOOD GUERNSEY NO. 87
Average production 6345.7 pounds of milk and 320.16 pounds of fat

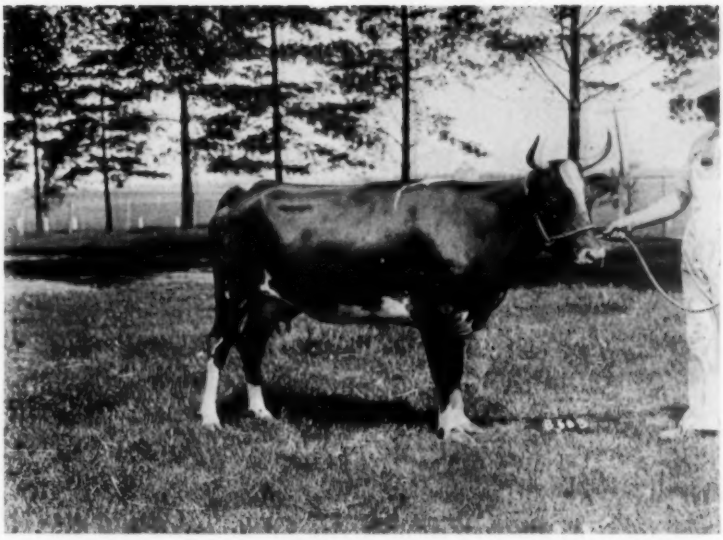

FIG. 7. SCRUB COW NO. 60

Average production 3313.2 pounds of milk and 178.47 pounds of fat

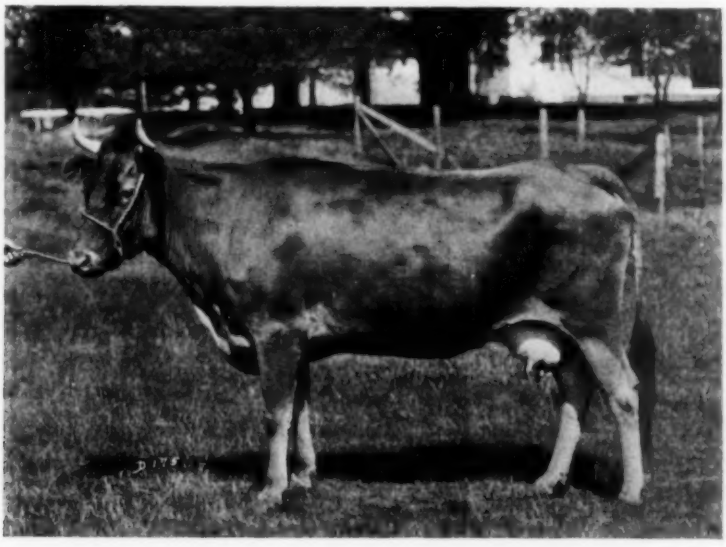

FIG. 8. HALF-BLOOD JERSEY NO. 241, OUT OF SCRUB NO. 60

Average production 6137.9 pounds of milk and 349.42 pounds of fat

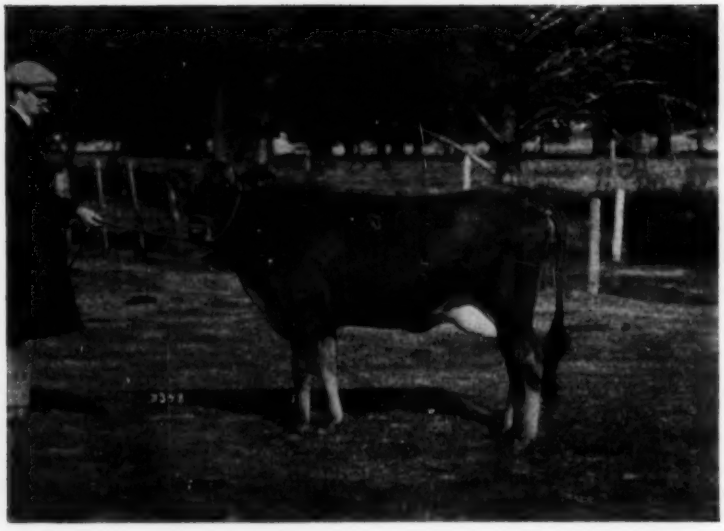

FIG. 9. THREE-QUARTER-BLOOD JERSEY NO. 348, OUT OF HALF-BLOOD JERSEY NO. 241

Average production 5366.9 pounds of milk and 278.70 pounds of fat

CORN STOVER SILAGE VERSUS CORN SILAGE FOR MILK PRODUCTION[1]

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The information available on the feeding value of corn stover silage is limited and no experimental data has been reported comparing corn stover silage, made from cured corn stalks, with corn silage, as feeds for milk production.

Rusk, of the Illinois Experiment Station, successfully maintained breeding beef cows through the winter on corn stover silage, and one pound of cotton seed meal daily. Long, of the Missouri Station reports that breeding beef cows gained in weight on a ration of corn stover silage and wheat straw.

In Wisconsin very little of the corn is husked as standing corn. The stalks are needed for feed and are usually harvested with the grain to be made into silage or are fed separate in the dry form. Claims have been made that corn stover silage, that is the stalks with ears removed, is equal in feeding value to ordinary corn silage. It was the purpose of the experiment reported here to obtain information that would corroborate or refute these claims, with reference to milk production.

ANIMALS USED

Eight cows producing an average of about one and one-fourth pounds fat daily at the time the trial was started, were used. They were separated into two equal groups.

PERIODS

Two periods each of four weeks duration with a preliminary week preceding each. The double reversal method of feeding the two kinds of silage was used.

[1] Read at Animal Production Society, Chicago, Ill., December, 1920.

FEEDS USED

Concentrate mixture:

	parts
Ground corn	4
Wheat bran	4
Linseed meal	1
Cottonseed meal	1

Equal amounts of concentrates fed each lot.

Alfalfa hay fed at the rate of slightly less than one pound of hay daily to each 100 pounds of cow weight. Equal amounts of hay fed each lot.

SILAGE

Each kind of silage was fed ad libitum twice daily. The corn stover silage tended to be somewhat dry near the wall of the silo. A good average sample of the silage contained 73 per cent of water. The cows took to the stover silage readily, although they consumed on an average of about 5 pounds less daily per animal than of corn silage.

RESULTS

The average results for the two periods for each ration are shown in the following tables:

TABLE 1

AVERAGE RATION	AVERAGE DAILY YIELD		TOTAL FAT	LOSS IN LIVE WEIGHT
	Milk	Fat		
	pounds	pounds	per cent	pounds
Corn silage ration Corn silage 33.25 pounds Alfalfa hay 8.78 pounds Concentrates 9.39 pounds	27.4	1.05	3.98	156
Corn stover silage ration Stover silage 28.31 pounds Alfalfa hay 8.78 pounds Concentrates 9.39 pounds	24.5	0.98	4.00	110

It will be noted from the above data that the average daily milk production on the corn silage ration was about three pounds more daily per cow than on the corn stover silage ration. A

corresponding relationship existed also in the productions of butter fat. Both lots lost some in live weight. The total loss of weight on the corn silage ration exceeded that on the corn stover ration by 46 pounds. This difference is not great enough to be considered significant.

TABLE 2

Financial summary

Corn silage ration:	
2103.9 pounds grain at $50.00 per ton	$52.60
7448.0 pounds silage at $6.50 per ton	24.20
1964.0 pounds alfalfa at $30.00 per ton	29.46
Total	$106.26
Corn stover silage ration:	
2104.2 pounds grain at $50.00 per ton	$52.60
6352.0 pounds silage at $4.00 per ton	12.60
1964.0 pounds alfalfa at $30.00 per ton	29.56
Total	$94.76

TABLE 3

Feed cost per hundred weight of milk and pound of butter fat

	COST OF MILK PER HUNDRED WEIGHT	COST OF BUTTER FAT PER POUND
Corn silage ration	$1.73	$0.43
Corn stover ration	1.73	0.43

The data shows that the costs per hundred weight of producing milk and per pound of butter fat are the same when the prices applied to corn silage and corn stover silage per ton, bear respectively the relation of $6.50 to $4.00. In other words the price at which the corn stover silage could be figured was 61 per cent of the price at which corn silage was valued. It is planned to carry on a more extensive trial than the one here reported at an early date.

THE ESTIMATION OF BUTTER FAT IN CREAM

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AND

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Senior Food Chemist City Health Department, Baltimore, Maryland

It has been stated that one of the greatest difficulties confronting the accurate estimation of butter fat in cream lies in the problem of securing a fair and representative sample. While we readily agree with this observation it has been the product of our investigation to conclude that an even greater source of error arises in an extension of the problem of sampling, namely, in the lack of uniformity which presents itself in many cream samples.

Analysts engaged in the routine sampling and testing of cream are, no doubt, well aware of the churning which occurs in commercial cream during transportation. This phenomenon, which is essentially a coagulation of fat globules, is due primarily to the effect of agitation. Higher stages of acidity favor its development and consequently it is during the warmer months that a greater number of churned shipments are found. However, since it is possible to have a certain measure of the conditions which favor the development of churning during all seasons it is not particularly unusual to encounter it at any time during the year. There are, furthermore, different degrees of churning; it may vary from a condition requiring the closest scrutiny to detect to its more familiar aspect in which large particles of fat can be recognized on the surface of the cream.

The effect of churning upon the accuracy of a test will depend principally upon two factors, the degree of churning and the amount of sample which is used for analysis. The application of this assertion will at once occur to those who have had occasion to sample and test creams which have developed a marked degree

of churning. It is impossible to rely on individual results and it is only by averaging the figures from a large number of tests that the effect of the unevenness is minimized to such a degree that it is practically negligible. The same purpose is served by using a large quantity of sample in such cases. With samples which are churned to a lesser extent the influence is, of course, correspondingly less upon individual results and with entirely homogenous samples the accuracy will depend more upon the precision of the method and skill of the analyst than upon the condition of the sample.

In the testing of cream it is usual to employ one of the extraction processes or the Babcock method. Of the extraction processes, the Roese-Gotleib method is perhaps more widely used than any other method. This method employs from 1 to 2.5 grams of sample. Some tests direct the use of from 3 to 5 grams, but we have found that the use of more than 2.5 grams, particularly of heavy cream, is likely to result in an incomplete extraction of fat by the amount of solvents called for in the several extractions. With the smaller amounts of sample we have had no trouble in completing the extraction in all cases.

A tabulation of our results with this method shows that in ten duplicate fat tests made during the summer months (from August 20 to September 2) the average variation was 1.18 per cent. The greatest single variation was 4.18 per cent and there were a number of duplicate tests which varied more than 2.00 per cent. During the fall (from September 3 to December 16) the average variation of 30 duplicate tests was 0.5 per cent, while a series of 69 duplicate tests made from December 16 to February 23 showed a variation of 0.11 per cent. The greater variation during the summer months was due to the greater degree of partial churning which occurs during this season.

These results indicated rather forcibly the limited application of this method to the field of routine cream testing, a failure which we attribute solely to the difficulty of securing homogenous samples. It is regrettable that there is no convenient apparatus or method by which it would be possible to homogenize samples before analysis. Our experience in testing homogenous samples

of cream has been entirely satisfactory and we have every reason to believe that a preliminary procedure such as we suggest would effectually solve the problem of accurate routine work with this method.

By reason of the larger portion of samples employed in analysis together with its other features of speed and economy, the Babcock method suggests itself at this time as a subject of investigation. We recognized the limitations of its precision when testing high-fat creams but on the other hand when testing partially churned samples its accuracy in representing the fat content of entire consignments as compared with the extraction method appeared to be much greater.

Accordingly, a number of comparative tests were conducted. It was decided to use a 24-bottle steam driven Babcock machine with a speed of about 800 revolutions per minute; a 36-bottle electrically driven machine with a speed of about 1000 revolutions per minute and to compare results with those of the extraction method. By employing only homogenous samples it was possible to use the results obtained by the extraction method as a standard. In the following comparisons we were careful to select only those results in which duplicate determinations by this method checked closely.

The Babcock tests were also made in duplicate, employing an accurately weighed 9-gram sample. After first diluting the 9-gram sample with an equal volume of water the usual procedure for carrying out this test was followed. The fat columns were entirely clear in those cases in which the results were used for comparisons. The Babcock bottles used were of the type which have a bulb in the stem, and are graduated in 0.2 per cent, with a capacity of 25 per cent.

The comparative results are as follows:

NUMBER	EXTRACTION METHOD	ELECTRIC BABCOCK MACHINE	STEAM BABCOCK MACHINE
1	36.60	37.20	38.40
2	40.61	40.70	42.00
3	36.75	39.40	39.20
4	43.84	45.60	45.20
5	36.32	36.40	37.00

It was interesting to note from these figures that although in no case did the three methods check there was nevertheless a characteristic relationship between them. The figures of the extraction method were invariably lower than those by either Babcock process, and the electrically-driven machine gave results which were, as a whole, lower than the steam-driven machine.

In spite of the accredited accuracy of the extraction method and the reliance which we had gained through experience with homogenized samples several additional ether extractions were made in some of the tests. In all cases they failed to show any appreciable difference in the results and thus eliminated the consideration of incomplete extractions. As an additional check it was decided to compare the results of fat tests by this method with the results obtained by determining the total solids, and, after a complete mechanical removal of the fat, the solids-not-fat. The difference between the first and the latter figure gave a fat percentage which approximated the direct fat extraction within an acceptable limit. At least, it served the purpose of showing that the extraction method was more nearly correct than either Babcock process. By reference also to tables showing the computed value for the solids-not-fat content of cream with varying percentages of fat it was possible to further check the relative accuracy of the extraction method.

In seeking to explain the difference in the results obtained by the two Babcock methods a careful study of all factors concerned showed that the sole difference was in the advantage in speed possessed by the electric machine, as before stated, an advantage of about 200 revolutions per minute.

A review of the text books and other publications upon the subject indicated that with machines of our type a minimum speed of about 700 to 800 revolutions per minute was essential to accurate results while directions regarding the maximum speed where rather indefinite, about 1200 revolutions per minute being the highest speed that was noted. In other words, while it was agreed that a certain minimum speed was necessary to bring all of the fat to the top, it was also indicated that a speed much less than this minimum would not accomplish this end

but would result in readings correspondingly low. The higher speeds were not mentioned except as permissible maxima and in no case was any reference made to the influence of such speeds upon the final result.

Nevertheless, upon the assumption that the greater centrifugal force possessed by the electric machine did have some influence upon the fat column, further comparative tests were made at a speed of about 1200 revolutions per minute.

The results of these tests may be tabulated as follows:

NUMBER	EXTRACTION METHOD	ELECTRIC BABCOCK MACHINE
1	38.54	38.80
2	43.49	44.40
3	38.38	39.30
4	34.60	34.80
5	37.73	38.10
6	36.58	37.30

By comparison with the first series of tests it was found that the average variation under the increased speed had dropped, from 1 per cent to about 0.60 per cent which quite clearly demonstrated that the additional centrifugal force alone was the responsible agent in effecting the change in variation of results.

In studying the influence of the additional force upon the fat readings it appeared, in the first place, quite obvious that with the least speed employed in these experiments (800 revolutions per minute with the steam-driven machine) the force had been sufficient to accomplish the purpose of bringing all of the fat within the graduated stem of the Babcock bottle. In fact, our findings lead us to the opinion, which we believe has not been previously expressed, that any speed above that minimum necessary to bring all of the fat to the top exerts a correcting influence upon the length of the fat column.

In all of the experiments with the Babcock method we were careful to record only those readings which showed an absolutely clear fat column, consequently we were prepared to reject the possibility of the variation being due to the presence of any solid

particles in the fat. There remained then for consideration the possible occlusion of water or acid, or perhaps both by the fat.

A final series of experiments were conducted with a view of determining this point. We had installed for this purpose a Babcock super-centrifuge, a machine with a guaranteed speed of 1800 revolutions per minute. This machine was operated at a speed of 1600 revolutions per minute, however, during these experiments. The following comparative results were obtained.

NUMBER	EXTRACTION METHOD	ELECTRIC BABCOCK MACHINE
1	33.50	33.60
2	45.18	45.20
3	40.83	40.80
4	39.12	39.20
5	37.49	37.40
6	44.83	44.80
7	40.02	40.00

The difference in the average of the above results is 0.01 per cent, while the greatest single variation is 0.10 per cent.

From the foregoing experiments with the Babcock method as applied to cream testing we believe that we have brought out a new conception of the purpose of centrifugal force. It has been thought, in the testing of both milk and cream, that a centrifugal force sufficient to bring all of the fat within the graduated stem of the Babcock bottle was all that was necessary to produce accurate results. We feel that our experiments have demonstrated the necessity of a force sufficiently powerful to not only accomplish this but in addition to render the fat free of any occluded liquid.

The last series of comparative tests we believe were important in demonstrating that by applying sufficient centrifugal force in the operation of the Babcock method for cream testing it is a relatively easy matter to produce results which compare favorably with those of the ether extraction method.

In concluding, it might be added that we believe that under the conditions now existing the Babcock test is the more suitable method for the purpose of routine cream testing. By employ-

ing a larger portion of sample it is more representative and consequently more accurate with cream samples of varying degrees of uniformity and through its speed and economy it is always possible to heighten the precision of the results by carrying out simultaneously a larger number of tests than can be conveniently done by the ether extraction method as is usually applied to cream testing.

SUMMARY

From the above investigation we would conclude that:

1. The ether extraction method does not give accurate results when applied to samples of cream which are partially churned, consequently it is not suitable for the general routine work of commercial laboratories.

2. The Babcock method has the advantage of minimizing the inaccuracies introduced by the lack of uniformity frequently encountered in cream, by reason of the use of a larger portion of sample in analysis.

3. However, the Babcock method as it is usually employed for cream testing gives high results. We attribute this to the occlusion of water or acid within the fat column.

4. By applying sufficient centrifugal force the fat column can be freed of the foreign liquid. When this has been accomplished the results obtained by this method compare favorably in accuracy and precision to those of the extraction method.

5. Under the present difficulties of obtaining uniform samples we believe that the Babcock method when properly carried out is to be preferred to the ether extraction process as a routine procedure.

THE ACCURACY OF BACTERIAL COUNTS FROM MILK SAMPLES[1]

R. S. BREED AND W. A. STOCKING, JR.

INTRODUCTION

At the present time all of the market milk sold in New York state is graded on a system based upon the number of bacteria present in the milk. Likewise an increasing amount of milk is being bought from dairy farmers on the same basis. This makes it important to all concerned that the methods of analyses used in the grading should be sufficiently accurate to justify the use made of them.

Because of questions that have been raised in regard to the matter, a series of coöperative analyses of milk samples have been made, the results of which are discussed in the present article.

PREVIOUS STUDIES

Because of the importance of the matter, numerous series of comparative analyses have previously been made in many of the public and private bacteriological laboratories in New York State; but the primary purpose of the majority of these tests has been merely to determine whether different analysts, working in the same or in different laboratories, could secure duplicate agar counts which were in close agreement.

The results secured have been such as to cause the majority of bacteriologists to feel that reasonable agreement could be

[1] Condensed from Technical Bulletin 75 issued by the New York Agricultural Experiment Station, Geneva, N. Y., and by the New York State College of Agriculture, Ithaca, N. Y. The analyses on which the report is based were made by R. S. Breed, J. D. Brew, H. J. Conn, W. D. Dotterrer and G. L. A. Rueble from Geneva; and A. M. Besemer, H. M. Pickerell, T. J. McInerney, and G. C. Supplee from Ithaca. Detailed analytical data will be found in the original bulletin, copies of which may be obtained on application to either institution.

obtained where the counts were made by a standard, uniform technique by trained analysts. Yet it has become increasingly evident that carelessness or slight modifications of essential procedures may produce widely divergent counts. The most extensive of the recent series of analyses is that made under the supervision of the late Prof. H. W. Conn (1). Other recent series are those discussed by Hatfield (2) and by Kilbourne (3).

It should not be forgotten, however, that the securing of closely comparable counts of bacteria from duplicate samples by different analysts where but a single method of analysis is used *does not prove that the counts obtained are an accurate or even a reasonably accurate count of the actual number of bacteria present.* It is easily possible that such counts are affected by a constant source of error which affects all duplicate counts proportionately. Or it may even be possible that variable errors completely destroy the accuracy of the counts even when there is no indication of their presence in the final counts.

For example, in the case of agar plate counts from milk, constant errors in count would be produced if all bacteria in milk existed in clumps of a constant average size. Such a condition would not be indicated in the counts as all would be reduced proportionately. Thus if the average clump contained two individuals, all counts would be reduced to one-half of the number of bacteria actually present. It may justly be argued that such an error would have no practical significance because its presence would be already discounted in the standards established for each grade of milk.

The great uncertainty in the present situation does not arise because it is thought that errors of this type exist; but because microscopic examination has shown that the average size of the clumps of bacteria in milk is not constant. In such a situation, the results are, undoubtedly, liable to inconstant errors of such a type that they pass undetected in comparative series of analyses made by the agar plate method. Thus, while all of the counts made from one lot of milk may be reduced to one half their true size by the presence of clumps containing an average of two individuals, another set of duplicate counts may be reduced to

one-third of their true size, another to one-sixth, another to one twenty-fifth, etc.

It is this condition of affairs that has made the development of a second, fundamentally different, method of counting bacteria in milk doubly important. Microscopic methods of counting have now been so perfected that it is possible not only to secure data regarding the actual size of the clumps of bacteria in milk, but also to make comparative analyses of a series of milk samples both by the usual agar plate method and by direct microscopic examination. This permits us to study the accuracy of the counts in a manner not heretofore possible.

Already extended series of comparative analyses have been made and published (4), (5). The analyses mentioned have, however, been largely the work of a single analyst, and are subject to the "constant" and "personal" errors which are always possible where analyses are made by a single person. Some preliminary comparative counts by several analysts from different laboratories were made in the series of counts discussed by Conn; but these were preliminary in nature, and, because of the difficulties involved in continuing the work where the laboratories were so widely separated, the work was discontinued.

For these reasons, the authors of the present paper in 1915 drew up a plan of investigation under which a series of samples of milk were to be analyzed in each of two laboratories maintained by New York State. A preliminary account of the results obtained has already been published in this Journal (6).

DESCRIPTION OF THE METHODS OF ANALYSES USED

In considering the results secured, it should be kept in mind that the counting of bacteria in milk is an arithmetical problem which would be no more difficult than counting the number of beans in a bag, the number of trees in a wood, or the number of seeds in a bushel of mustard seed, if the bacteria could be seen and handled as readily as these objects. However, bacteria are such tiny things that they can be seen only with high magnification, and they may occur in such incredible numbers that

comparisons with beans, trees or even mustard seeds give an inadequate idea of the difficulties involved.

Every one realizes the physical impossibility, or better, the impracticability of counting the trees on 100,000 acres of woodland. It is not only impracticable but physically impossible actually to count the bacteria in a quart of milk or even in a cubic centimeter of milk. In counting bacteria, as in counting trees, recourse must be had to the making of *estimates* of numbers not actual *counts*. So-called bacterial "counts" are not counts in the strict sense of the word, and like other estimates, their accuracy is largely dependent upon the care with which they are made. In order that the attention of the reader may be called to the fact, the word "count" has been placed in quotation marks throughout the body of the paper wherever it is used in the sense of an estimate.

Microscopic methods of counting bacteria. The simplest method of counting objects is by direct observation. In the case of bacteria this is obviously impossible because they are too small to be seen with the unaided eye. However, from the time when Leeuwenhoek (1683) first saw bacteria under his simple microscopes, microscopists have made more or less accurate estimates of the number of bacteria in various substances by direct observation under the microscope.

It is impossible to state who first made estimates of numbers of bacteria in milk by microscopic examination, but it is certain that no extensive use has been made of such methods until recently. (See Breed and Brew (7) for a detailed discussion of the historical development of these methods.)

Certain things prevent making the microscopic counts with absolute accuracy. These may be briefly summed up as follows:

a. There are certain optical limitations which make it difficult to prepare definite measured quantities of milk in such a way as to make all of the bacteria show with sufficient distinctness to permit counting under a microscope.

b. It is impossible to measure with absolute accuracy the exceedingly minute quantities of milk (1/500,000 to 1/300,000 cc.) which are the largest amounts that can be examined satisfactorily at any one time.

c. It is impossible to tell with certainty whether the bacteria seen under the microscope were actually living when the preparation was made.

d. Care must also be taken to prevent the growth and consequent increase in number of bacteria during the time consumed in preparing the material for microscopic examination, and to prevent the introduction of bacteria from extraneous sources.

Some of these difficulties can be partially, or even almost completely, overcome, but as a whole they can never be entirely eliminated by the most skillful analyst.

Agar plate methods of counting bacteria. The second method of counting bacteria under observation is fundamentally different from the first in that it involves inoculating a transparent nutrient jelly with measured quantities of milk containing bacteria. The nutrient jelly containing the bacteria is then incubated until the original bacteria have grown into masses of bacteria which are counted with a low power magnifying lens. These masses are usually termed "colonies" and it is these which are actually counted.

Such a method of counting may be compared to a method of counting seeds in which a measured quantity of seeds are germinated on a carefully prepared area of ground and the counts made from the plants which develop.

Historically, this method of counting bacteria is a direct outgrowth of the use of gelatin as a means of isolating pure cultures of bacteria first introduced by Koch in 1881. In the milk work which has been so extensively developed in the United States, agar has supplanted gelatin as a culture medium, and the method of making counts has been standardized largely through the efforts of members of the laboratory section of the American Public Health Association (8).

A modification of this technique, called the "little plate" method, has recently been developed and advocated for use by Frost (9). This differs from the technique ordinarily used in control laboratories in that the colonies which develop in agar are examined and counted under a compound microscope before they are visible to the unaided eye. Thus the time ordinarily

consumed in waiting for the development of colonies is greatly shortened. In general, this technique has the same advantages as that possessed by the usual agar plate method, and is subject to the same general type of limitations.

These limitations upon the culture methods of counting bacteria are, as in the case of the limitations upon the microscopic technique, such that it is impossible to secure absolutely accurate counts. The chief difficulties may be summed up as follows and may be better understood if the comparison with the planting of seeds on prepared soil is kept in mind.

a. Only those bacteria grow which survive the necessary manipulations and are capable of growth into visible colonies under the conditions of aeration, food, moisture, temperature, etc., which are present. It is never certain that all of the bacteria originally living in the milk have grown and formed countable colonies on the plates.

b. Overcrowding of the colonies on the agar may prevent the development of some bacteria, or single bacterial colonies may grow so rapidly that all other colonies are repressed. The latter, so called "spreaders," are readily recognizable and their presence may be discounted. Likewise repression of colonies through overcrowding is frequently recognizable.

c. A third difficulty is still more fundamental. It arises because the bacteria frequently exist in milk in clumps of twos, threes, fours, or even larger masses. Since these clumps cannot be perfectly separated into their component individuals by any known method of shaking or manipulation, the culture medium is always seeded with many groups of bacteria. As these grow they form a single mass or colony, indistinguishable from colonies which have arisen from single individuals. Counts of colonies are therefore never comparable to counts of bacteria, except in those cases where the bacteria exist in the original milk as isolated individuals.

d. The introduction of extraneous bacteria and increase in number of bacteria after the samples are collected and before the culture medium is inoculated is always possible, even where carefully controlled. As additional colonies thus introduced

cannot be detected from an examination of the plates, this always remains as a possible source of error.

e. Since it is impossible to get the best results where more than 300 colonies are grown on each plate, it becomes necessary to use minute quantities of milk (1/100,000 to 1/1,000,000 cc.) when accurate results are desired from samples giving "counts" in excess of 3,000,000 per cubic centimeter. While this measurement is made by the dilution method (admittedly a very accurate method of measurement), yet inaccuracies of measurement are known to occur.

Skill and care may reduce many of these possible sources of error to a minimum, but, as a whole they cannot be entirely eliminated.

Comparison between microscopic and agar plate methods of counting. Since it is impossible to make counts of bacteria that are known to be absolutely accurate, it becomes very difficult to determine whether more accurate counts can be made by direct observation than by cultural methods or whether the opposite condition holds true. This being the case, it is evident that the common assumption that the agar plate method gives the more accurate results has no real basis upon which to rest.

One point of difference between the two methods should always be kept in mind in considering the accuracy of results. Because of the fact that estimates made from plate counts must be based on a count of colonies, which should not exceed 300 per plate, the amount of milk examined is largest where the bacteria are few in number and continually grows smaller as the number of bacteria increases. It is as if all scattered trees on 10,000 acres of cleared land could be counted, whereas in making estimates of a similar area of woodland only one-tenth of an acre could be examined. Consequently it is probable that both the absolute accuracy and the percentage accuracy of plate counts are at their best in milk containing few bacteria, and that both the absolute and percentage accuracy of results decreases as the number of bacteria increases.

In contrast to this, microscopic counts are made from a small but fairly constant amount of milk regardless of the number of

bacteria present. It is as if one acre plats from 100 different places selected at random could be examined in counting the trees upon 10,000 acre plats regardless of the number of trees present. Under these conditions, the percentage accuracy is at its best, and probably also the absolute accuracy, under intermediate conditions. Where there are few bacteria present, the percentage accuracy becomes poor though, for practical purposes in grading milk, this is of little consequence because the absolute error is not large.[2] A failure to appreciate these differences in the two methods of counting has caused some unjustified criticism of microscopic counts.

PLAN OF THE PRESENT INVESTIGATION

The first work done was to analyze a series of twenty samples of fresh milk of a miscellaneous character (known in this article as series A). Counts were made from these both by the agar plate method and by direct microscopic examination. Chiefly because of the inexperience of some of the analysts with microscopic counting, and because the miscellaneous character of the flora introduced so many possible sources of error that it was impossible to determine the exact cause of particular variations, the results obtained did not throw as much light on the question of the accuracy of the counts as had been expected.

On the other hand, the results from one of the samples which was known to contain a predominant colon flora were so instructive that it was decided to use samples of this type for further work. A second series of samples (series B) inoculated with the colon organism was therefore analyzed on April 10, 1916, and later still (February 7, 1917), a third series (series C) was examined. As the results obtained from series B and C were instructive and explanatory of the results from series A, the former series (B and C) are discussed first.

[2] The meaning of absolute and percentage accuracy as here used can be made clearer by a simple example. The percentage difference between 1000 and 2000 is the same as the percentage difference between 1,000,000 and 2,000,000; but the absolute difference in the first case (1000) is much smaller than the absolute difference in the second case (1,000,000).

SERIES B. SAMPLES INOCULATED WITH AN ORGANISM OF THE COLON GROUP

The advantage of the colon organism for the purpose in hand rises from the fact that it tends to live in milk as isolated individuals, only occasionally forming clumps of two, four or rarely more individuals. Likewise, it is always found evenly distributed throughout microscopic preparations of milk whenever it is present. It also grows well on ordinary media and at ordinary incubation temperatures. Thus "counts" carefully made from milk containing this organism may be expected to be nearly free from the sources of error previously discussed.

In order to have a series of checks and counter checks upon the accuracy of the results, it was decided to use but three batches of milk, each batch to be analyzed in triplicate by each analyst. The three batches were in each case prepared from a liter of a high grade, freshly-drawn milk known to contain very few bacteria. The first liter was inoculated with 2.5 cc. of an actively growing culture of an organism of the colon group, the second liter with 5 cc., and the third liter with 10 cc. of the same culture. Thus it was expected that the final "counts" would show ratios of approximately 1:2:4. From a rapid microscopic examination of the first liter of milk it was expected that the final "counts" from this milk would be between 100,000 and 500,000 per cubic centimeter, and directions for making dilutions were given accordingly.

Ten samples, each containing about 15 cc. of milk, were sent to each of six analysts. No. 1 was a sample of the high grade fresh milk used as a base in preparing the inoculated milk. Nos. 2, 3 and 4 were samples from the liter of milk containing the smallest amount of the colon culture. Nos. 5, 6 and 7 were from the second liter of inoculated milk, while nos. 8, 9 and 10 were from the liter of milk containing the largest number of colon organisms. This distribution of samples resulted in eighteen separate analyses of each of the three liters of inoculated milk by two different methods, a total of 108 analyses. A further check on the final results was established by having all petri

dishes prepared in triplicate from each of two dilutions. The plates were recounted in each case by a second analyst in order that any carelessness in counting might be eliminated. Likewise, the microscopic preparations were made in duplicate from each sample, and they were also recounted by a second analyst. The six sets of samples were prepared at Geneva at 7.00 a.m., were thoroughly iced, and three sets forwarded by messenger to Ithaca. The analytical work was started in both laboratories about 10.00 a.m., and the petri plates and microscopic slides were prepared in all cases before noon. In one respect the samples used in this series were not perfect as later examination showed that masses of bacteria, which did not break apart readily, had formed in the thin film of cream on the skim milk culture. Consequently, occasional groups of 10, 20 or more individuals were found in the final microscopic preparations.

To facilitate comparisons between the results secured by the two different methods, standard deviations and coefficients of variability have been computed for the estimates of numbers per cubic centimeter as reported for each sample; but no further mathematical analysis of the data has been made.

a. Agar plate counts

Technique used. The analysts were instructed to prepare dilutions as follows: Sample 1—1:10 and 1:100; samples 2, 3 and 4—1:1,000 and 1:10,000; samples 5 to 10—1:10,000 and 1:100,000. These were made by using freshly prepared and accurately measured 9 and 99 cc. water blanks. One cubic centimeter pipettes were used by all analysts, but those used at Ithaca were all of the type with two graduation marks, while those used at Geneva were of the type with a single graduation mark. As the latter were supposed to have been calibrated to *deliver* 1 cc. quantities, analysts B and D merely emptied them carefully in each case as used, and did not rinse them by drawing the dilution water into the pipette.

All analysts were supplied from a single lot of agar (1 per cent Difco peptone, 1 per cent lactose, 0.3 per cent Liebig's beef

extract, 1.5 per cent air dried agar) prepared at the Ithaca laborratory. The acidity of this agar, before adjusting, was 1.6 per cent normal to phenolphthalein, and by the addition of NaOH it was reduced to 1.1 per cent. No record of the H-ion concentration was made.

Incubation temperatures were not definitely controlled in all cases, but they were between 25° and 30°C. Plates were counted at the end of three days when the colonies were well developed. Troublesome spreaders were practically absent, and recounts of the colonies on the plates were in all cases in close accord with the first counts.

The "counts" for samples 2, 3 and 4, as reported here, were secured by averaging the counts from the individual plates from both dilutions; while the counts from the 1:100,000 dilutions were usually discarded for the remaining samples, as they ordinarily showed less than 20 colonies per plate.

Counts obtained. A summary of the results are given in table 1. The individual analysts obtained very regular results as indicated by the standard deviations and coefficients of variability given in table 3. The coefficients of variability ranged from 7.7 for samples 2, 3 and 4 to 13.8 for samples 5, 6 and 7 with an average coefficient of variability of 11.1.

The tabulation of results revealed a tendency of the part of the Geneva men (B, C, and D) to report lower "counts" than those reported from Ithaca, a tendency which had been previously noted in series A. Thus in series B, only nine out of 27 "counts" from the Geneva laboratory were higher than the final averages, while 20 out of 27 "counts" from the Ithaca laboratory were higher than the average figures.

As the differences were relatively small, and as one of the Geneva men (C) who rinsed his pipettes obtained higher counts than the others, a suspicion arose that they were caused by inaccuracies in the measurements of the milk and of the dilution waters. Consequently, the 1 cc. pipettes used were recalibrated, whereupon it was found that the use of the one mark pipettes without rinsing in the dilution waters was causing a small but

TABLE 1

Series B. Summary of plate and microscopic "counts"

SAMPLE NUMBER	ANALYST*	PLATE "COUNT PER CUBIC CENTIMETER"	ANALYST*	GROUP "COUNT PER CUBIC CENTIMETER"		INDIVIDUAL "COUNT PER CUBIC CENTIMETER"	
				Average for each analyst	Combined average	Average for each analyst	Combined average
1		390			3,000		4,000
2–4	BB	325,000	BB	†262,000	282,000	†499,000	494,000
	BD		BD	303,000		489,000	
	CC	375,000	CA	275,000	241,000	430,000	405,000
	CB		CB	206,000		381,000	
	DD	323,000	DD	291,000	259,000	435,000	405,000
	DC		DA	227,000		376,000	
	FF	378,000	FF	301,000	343,000	385,000	562,000
	FI		FI	386,000		739,000	
	HH	330,000	HH	211,000	216,000	276,000	293,000
	HF		HF	221,000		310,000	
	II	347,000	II	293,000	220,000	773,000	731,000
	IH		IH	147,000		690,000	
Final av....		346,000			260,000		482,000
5–7	BB	558,000	BB	†559,000	585,000	†1,018,000	993,000
	BD		BD	611,000		969,000	
	CC	650,000	CA	549,000	490,000	852,000	803,000
	CB		CB	432,000		755,000	
	DD	602,000	DD	640,000	582,000	1,097,000	948,000
	DC		DA	525,000		798,000	
	FF	621,000	FF	545,000	550,000	816,000	787,000
	FI		FI	556,000		758,000	
	HH	658,000	HH	411,000	442,000	617,000	660,000
	HF		HF	473,000		702,000	
	II	754,000	II	609,000	432,000	865,000	689,000
	IH		IH	255,000		513,000	
Final av....		640,000			514,000		813,000
8–10	BB	1,410,000	BB	†1,127,000	1,165,000	†1,919,000	1,946,000
	BD		BD	1,204,000		1,974,000	
	CC	1,170,000	CA	1,083,000	982,000	1,686,000	1,584,000
	CB		CB	882,000		1,483,000	
	DD	1,205,000	DD	1,513,000	1,339,000	2,475,000	2,091,000
	DC		DA	1,166,000		1,708,000	
	FF	1,551,000	FF	822,000	969,000	1,473,000	1,472,000
	FI		FI	1,116,000		1,472,000	
	HH	1,491,000	HH	567,000	791,000	969,000	1,240,000
	HF		HF	1,015,000		1,510,000	
	II	1,470,000	II	895,000	762,000	1,230,000	1,090,000
	IH		IH	629,000		949,000	
Final av....		1,383,000			1,001,000		1,570,000

* The first analyst named made the plates or slides. The second counted them.

† As Analysts A, B, and D examined 1 cu. mm. of milk in making their "counts," the exact number of groups, or of bacteria that they saw is obtained by dividing their results by 1000. The remaining analysts examined a little more than 1 cu. mm.

detectable loss as they were calibrated to *contain* 1 cc. and did not *deliver* the full amount.

In order to test the matter further, the Geneva analysts used the same pipettes for the next series of analyses, carefully rinsing

TABLE 2

Series B. Observed ratios between the counts from the 3 liters of inoculated milk. Theoretical ratio 1:2:4

ANALYST*	RATIO FOR PLATE "COUNT"	ANALYST*	RATIO FOR GROUP "COUNT"	RATIO FOR INDIVIDUAL "COUNT"
BB	1:1.70:4.30	BB	1:2.13:4.30	1:2.04:3.85
BD	1:1.74:4.39	BD	1:2.02:3.97	1:1.98:4.04
CC	1:1.72:3.12	CA	1:2.00:3.94	1:1.98:3.92
CB	1:1.76:3.13	CB	1:2.10:4.28	1:1.98:3.89
DD	1:1.96:3.93	DD	1:2.20:5.20	1:2.52:5.70
DC	1:1.77:3.54	DA	1:2.31:5.14	1:2.12:4.54
FF	1:1.59:4.06	FF	1:1.81:2.73	1:2.12:3.83
FI	1:1.69:4.15	FI	1:1.44:2.92	1:1.03:1.99
HH	1:2.03:4.31	HH	1:1.95:2.69	1:2.24:3.51
HF	1:1.95:4.71	HF	1:2.14:4.59	1:2.26:4.87
II	1:2.10:4.06	II	1:2.08:3.05	1:1.12:1.59
IH	1:2.25:4.43	IH	1:1.74:4.21	1:0.50:0.92
Average	1:1.85:4.00		1:1.98:3.85	1:1.69:3.28

* The first analyst named made the plates or slides. The second counted them.

TABLE 3

Series B. Summary of table 1

SAMPLE NUMBER	PLATE "COUNT" PER CUBIC CENTIMETER			GROUP "COUNT" PER CUBIC CENTIMETER			INDIVIDUAL "COUNT" PER CUBIC CENTIMETER		
	Final averages	Standard deviation	Coefficient variation	Final averages	Standard deviation	Coefficient variation	Final averages	Standard deviation	Coefficient variation
2–4	346,000	26,600	7.7	260,000	69,700	26.8	482,000	136,000	33.6
5–7	640,000	88,300	13.8	514,000	124,000	24.1	813,000	221,000	27.2
8–10	1,383,000	163,000	11.8	1,001,000	213,000	21.3	1,570,000	377,000	24.0
Average			11.1			24.1			28.3

each pipette as used. Likewise, in order to secure more accurate dilutions, 1:10 dilutions were prepared for each sample by withdrawing 5 cc. of milk with a 5 cc. pipette, and adding this to 45 cc. of sterile water. Subsequent dilutions were prepared with 1 cc. pipettes. The results secured with this improved system of measurement are discussed on page 60.

The ratios between the final average "counts" made by each analyst, as shown in table 2, agree well with the expected ratio of 1:2:4. It is worth noting, however, that in all but three of the twelve triplicate ratios, the first ratio is less than the 1:2 ratio which was expected. As the average ratio between the first and last "counts" was almost exactly 1:4, this condition raised the question whether the second liter of milk may not have failed actually to receive the full number of bacteria that were supposed to have been added. The final average ratio for all of the plate "counts" was 1:1.85:4.00.

The regularity of the plate "counts" and the agreement between the observed and the expected ratios make it probable that the figures given by the analysts were actually accurate estimates of the number of bacteria present. Yet it must be remembered, as already explained, that both of these things might be true in the case of agar plate "counts" which were actually very inaccurate.

All of the analysts agreed that Sample No. 1 contained very few bacteria, the agar plate "counts" being less than 800 per cubic centimeter in all cases. The final average "count" was 390 per cubic centimeter. The results show that this milk contained so few bacteria that the number present could not have influenced the final figures as reported for the remaining samples in the series.

b. Microscopic counts

Counts of two kinds were made by microscopic examination of the stained dried milk. The first of these, spoken of in this paper as the "group" count, was obtained by regarding each isolated organism and each actual clump of two or more organisms as single "groups." From theoretical considerations, it

was expected that the estimate based on this count would be lower than the agar plate "counts" as the latter are based on the growth of the "groups" after they are partially broken apart in the preparation of the dilution waters.

A count of individual bacteria was also made, any organism showing clear indication of approaching division being recorded as two individuals. It was expected that the estimate based on this count would in all cases be larger than the agar plate "count."

All analysts examined 100 fields of the oil immersion lens on each of two duplicate preparations from each sample, and nearly all recorded not only the number of bacteria seen, but also the number of twos, threes, fours, etc. Analysts A, B, and D, who were more experienced in the technique than the others, used special ocular micrometers ruled in circles and quadrants of circles, and adjusted the tube length of their microscopes so that the dried solids from 1/600,000 cc. of milk were visible in each microscope area counted. As only the central part of the field was used, the definition was clear and sharp and the danger of overlooking bacteria was lessened. Each of the three analysts mentioned examined 200 fields for each sample of milk, so that each counted the bacteria in 1/3000 cc. regardless of the number present.

The less experienced men used the entire field of the microscope and did not adjust their instruments so that it was necessary for them to use an irregular number in their computations. In all cases they examined a larger amount of milk than did the experienced men, but their counts were, nevertheless, decidedly less regular than those returned by the experienced men.

Table 1, last four columns, gives the average figures obtained for each of the three batches of colon inoculated milk. In this case both the original count and the recounts are given and these are then combined into a final average figure for each of the six samples.

Computations of the standard deviations are given in table 3 with their coefficients of variability. From this table it will be seen that the coefficients of variability of the group "counts"

vary from 21.3 for the group of samples containing the largest number of bacteria to 26.8 for the group of samples containing the fewest bacteria. The average for all samples was 24.1.

The coefficients of variability for the individual "counts" were larger (as was to be expected) and varied from 24.0 for the group of samples having the largest number of bacteria to 33.6 for the group of samples containing the fewest bacteria. The average for all samples was 28.3.

In spite of the variability of the microscopic "counts," the irregularities counterbalanced each other in such a way that the final average ratios, as given in table 2, are very close to the expected ratio of 1:2:4. Thus, for the group "counts" the observed ratio was 1:1.98:3.85, while that for the "counts" of individual bacteria was 1:1.69:3.26. It will be observed that all of these ratios are lower than the expected ratios. As there are several possible explanations for this condition, it is useless to speculate upon them.

Some fairly wide variations from the expected ratio will be found among the ratios actually obtained by the individual analysts which are recorded in table 2. However, many of the ratios obtained agree well with the expected ratio.

It should be pointed out in connection with these "counts" that neither agreement in results nor the fact that the observed and the expected ratios are on close agreement prove that the "counts" as given are truly accurate. These conditions raise a strong probability that the final average figures are very close approximations to the actual number of groups of bacteria present per cubic centimeter, but they do not *prove* the accuracy of the results.

c. Comparison between the plate and the microscopic counts

It is not until the results obtained by the two different methods of counting are compared with each other that the probable accuracy of the counts can be established with reasonable certainty. An examination of the results as given in table 1 shows that the final average group "counts" are invariably lower than

the final average plate "counts," while the final average individual "counts" are higher than either of the other counts. This is as it should be if the results were truly accurate, for it is to be expected that the groups were somewhat broken up in the process of preparing the plates, thereby causing more colonies to appear on the plates than there were groups originally present in the milk. However, it is not to be expected that all groups were broken into their component individuals, so that the plate "count" should never be as large as the individual "count" if both were truly accurate.

Even the average "counts" as reported by each individual analyst for each sample usually showed a plate "count" intermediate between the two microscopic "counts." As is to be expected, however, this relationship is not as perfectly maintained as it is in the case of the final average "counts." Thus in eight cases out of a possible 54, the plate "counts" reported were less than the group "counts" as reported, while, in 17 cases out of a possible 54, the plate "counts" exceeded the individual "counts." Although this analytical work was done with care, it is evident that errors in "counts" were not absolutely eliminated.

The test for accuracy just discussed is a relatively severe one, for it must be remembered that the milk used for analysis contained very small groups of bacteria (average size 1.6 individuals). This means that the individual "count" is only 1.6 times the size of the group "count" if both are perfectly made, so that large variations in the plate "counts" would cause these to be larger or smaller than the microscopic "counts." Since the conditions found indicate that all three counts represent fairly accurately the conditions as they exist, it then becomes possible, by a comparison between the group and the plate "counts" to determine how much the clumps were broken apart by the shaking in the dilution waters. Thus we find that, whereas the average group in the original milk from which samples 2, 3, and 4 were prepared, contained 1.85 individuals, the average group in the dilution water contained but 1.39 individuals. In the case of samples 5, 6, and 7 the average size of the group was

reduced from 1.58 to 1.27 by the shaking and dilution process; while in samples 8, 9, and 10 the average size of the groups was reduced from 1.58 to 1.13 individuals.

One of the most interesting comparisons which can be made between the three types of counts is that of their coefficients of variability. By examining these in table 3, it will be seen that the average coefficient of variability for all of the plate "counts" in series B was 11.1. On the other hand, the similar value for the group "count" is 24.1 and for the individual "count" it is 28.3.

This shows at once that the microscopic "counts" were more variable than were the plate "counts," a fact which is evident even on a casual inspection. There are certain things which should be kept in mind, however, in comparing these coefficients of variability, namely, that such variations may be produced by the limitations in the technique itself, by differences in the skill of the analysts, or by actual differences in the number of bacteria or groups of bacteria present in the quantities of milk examined by the different analysts.

The greater variability of the individual "counts" as contrasted with the group "counts" is, for example, due to the fact that it is always affected by one more source of variation than is the group count, namely differences in the number of individuals found in the groups. As both the group "counts" and the individuals "counts" were made from identical portions of the original samples of milk, the greater variability of the latter "counts" is caused by the fact that some analysts chanced to find larger sized groups than did others.

SERIES C. SAMPLES INOCULATED WITH AN ORGANISM OF THE COLON GROUP

As all of the analysts felt that the accuracy of the work thus far done could be still further improved as a result of the experience gained, it was decided to duplicate the last series of analyses, introducing such improvements in technique as had been suggested by the results secured.

Circumstances, however, prevented its repetition until February 7, 1917, at which time only four of the analysts who had participated in the work of both series A and B were available. The group of six was therefore completed by including one man who had participated in the work of series A only, and one man who had participated in the work of series B only.

Each analyst knew the general nature of the samples furnished, and knew something of the results as they were obtained in the laboratory in which he worked; but no comparisons were made between the "counts" as obtained in the two laboratories until the final reports were ready. These conditions were permitted as the work of every analyst was checked by a second analyst, and every man was keenly interested in finding the true state of affairs regardless of any preconceived ideas.

Six sets of samples were prepared at the Geneva laboratory. Three of the sets were sent to Ithaca by messenger as before, and three retained at Geneva. Sample 1 was taken from a high grade fresh milk which was used as the base for the inoculated samples. In preparing the three liters of inoculated milk for sampling, the procedure followed was to add enough of the colon culture to a liter of milk to give a count in excess of 1,000,000 per cubic centimeter as determined roughly by immediate microscopic examination. Then 500 cc. of this milk was diluted with 500 cc. of the uninoculated fresh milk to make a second liter of milk which presumably contained exactly one-half of the organisms present in the first liter. Then 500 cc. was taken from the second liter, and added to another 500 cc. portion of the uninoculated milk, making a third liter of milk which presumably contained one-half of the organisms from the second liter, or one-fourth of the organisms from the first liter. Samples 2, 3, and 4 were prepared from the third liter of milk; samples 5, 6, and 7 from the second liter; and samples 8, 9, and 10 from the first liter. Thus the final average "counts" from these three groups of samples were expected to show the ratio of 1:2:4 as before.

TABLE 4

Series C. Summary of plate and microscopic "counts"

SAMPLE NUMBER	ANALYST*	PLATE "COUNT PER CUBIC CENTIMETER"	ANALYST*	GROUP "COUNT PER CUBIC CENTIMETER"		INDIVIDUAL "COUNT PER CUBIC CENTIMETER"	
				Average for each analyst	Combined average	Average for each analyst	Combined average
1		623			5,500		7,250
2–4	BB	885,000	BB	†868,000	593,000	†1,187,000	813,000
	BC		BH	318,000		439,000	
	CC	784,000	AA	721,000	685,000	1,150,000	1,028,000
	CE		AI	650,000		906,000	
	EE	755,000	EE	783,000	674,000	1,297,000	1,075,000
	EB		EF	566,000		853,000	
	FF	842,000	FF	781,000	754,000	1,135,000	1,160,000
	FH		FE	728,000		1,184,000	
	HH	830,000	HH	451,000	680,000	642,000	935,000
	HI		HB	910,000		1,227,000	
	II	827,000	II	752,000	778,000	1,072,000	1,117,000
	IF		IA	804,000		1,163,000	
Final av....		821,000			694,000		1,021,000
5–7	BB	1,605,000	BB	†1,564,000	1,035,000	†2,238,000	1,493,000
	BC		BH	506,000		747,000	
	CC	1,470,000	AA	1,442,000	1,376,000	2,230,000	2,042,000
	CE		AI	1,310,000		1,854,000	
	EE	1,620,000	EE	1,519,000	1,368,000	2,468,000	2,152,000
	EB		EF	1,217,000		1,837,000	
	FF	1,715,000	FF	1,414,000	1,482,000	2,153,000	2,348,000
	FH		FE	1,550,000		2,543,000	
	HH	1,610,000	HH	864,000	1,333,000	1,275,000	1,806,000
	HI		HB	1,802,000		2,336,000	
	II	1,670,000	II	1,458,000	1,539,000	2,079,000	2,240,000
	IF		IA	1,620,000		2,400,000	
Final av....		1,615,000			1,356,000		2,014,000
8–10	BB	3,600,000	BB	†3,443,000	2,672,000	†4,796,000	3,862,000
	BC		BH	1,901,000		2,928,000	
	CC	3,710,000	AA	2,758,000	2,783,000	4,367,000	4,191,000
	CE		AI	2,808,000		4,016,000	
	EE	3,680,000	EE	3,148,000	2,947,000	5,086,000	4,594,000
	EB		EF	2,747,000		4,102,000	
	FF	3,620,000	FF	2,559,000	2,595,000	3,583,000	3,934,000
	FH		FE	2,630,000		4,285,000	
	HH	3,340,000	HH	1,750,000	2,772,000	2,781,000	3,895,000
	HI		HB	3,794,000		5,010,000	
	II	3,890,000	II	3,198,000	3,347,000	4,408,000	4,771,000
	IF		IA	3,496,000		5,134,000	
Final av....		3,640,000			2,853,000		4,208,000

* The first analyst named made the plates or slides, the second counted them.

† As all analysts examined 1 cu. mm. of milk in making these "counts," the exact number of groups, or of bacteria seen is obtained by dividing this figure by 1000.

a. Agar plate counts

Technique used. All of the agar used was prepared in a single batch at Ithaca and it had the same composition as before. The acidity of the medium was approximately 1 per cent normal acid to phenolphthalein, and was found to have a reaction of about pH = 7.6.

As already stated, the Geneva analysts were instructed to rinse all 1-cc. pipettes as they were used, and all analysts prepared their first dilutions by adding 5 cc. of milk to 45 cc. of sterile water. Instructions were given to use 1: 10 and 1: 100 dilutions for sample 1; 1: 1,000 and 1: 10,000 dilutions for samples 2, 3, and 4; and 1: 10,000 and 1: 100,000 dilutions for the remaining samples. Later it became evident that these instructions were satisfactory as plates were secured in each case which developed more than 30, and less than 300 colonies. The plates were recounted by a second analyst from the laboratory in which the plates were prepared.

Counts obtained. The average "counts" are given in table 4. From these it will be seen that the high grade milk was again found to have a very low "count," the final average plate "count" being 623 per cubic centimeter. Thus it was sufficiently free from bacteria to serve satisfactorily as a base for the inoculated samples.

The inoculated milk gave higher "counts" than before, the final average plate "count" from samples 2, 3, and 4 being 821,000 per cubic centimeter as contrasted with 346,000 per cubic centimeter for the similar group of samples from series B. With the single exception of the "count" obtained by analyst B for sample 7, the plate "counts" were in close agreement. In the instance mentioned the number of colonies which developed on the plates was much less than developed on the plates made from the duplicate samples by the same analyst, and likewise fewer than the number which developed on the plates made by the other analysts. As the duplicate plates from this one irregular sample agreed with each other, it was concluded that analyst B had inadvertently used a 99 cc. water

blank in place of a 9 cc. blank in preparing the original dilutions. If no results had been at hand for comparison, the error would have remained undetected.

Only minor and insignificant differences appeared in the recounts of colonies from the agar plates.

The tendency of the Geneva analysts to return a majority of the counts which were below the average was practically eliminated, as they returned but 15 out of a possible 26 counts which were lower than the average. On the other hand, the Ithaca analysts returned 12 out of a possible 27 counts which were lower than the average. This improved condition was probably associated with the rinsing of the one mark pipettes and the greater care taken in securing accurate measurements in making the dilutions.

The final average ratio between the plate "counts" was 1:1.97:4.43 (table 5). This is neither decidedly better nor worse than the similar ratio of 1:1.85:4.00 obtained in series B. The differences between the observed and expected ratios are small in both cases. Even the ratios as computed for the individual analysts are free from gross discrepancies. All agreed in reporting that samples 8, 9, and 10 contained more than four times the number of organisms found in samples 2, 3, and 4, making it probable that this was actually the case. In every instance, the ratio between the "counts" for samples 2, 3, and 4 and for samples 5, 6, and 7 was very close to the expected ratio of 1:2.

The maximum and minimum "counts" have been collected in a separate table (table 7). A comparison with the similar results from series B shows the results from series C to be more regular than those previously obtained. This fact becomes more evident from the computed standard deviations and their corresponding coefficients of variability which are recorded in table 6. From this table, it will be seen that the coefficient of variation for both the first and second groups of samples in series C was only 7.1. The similar value for the third group was 10.6, making an average coefficient of variability of only 8.3. This is even better than the average of 11.1 obtained in series B, and indicates that the improved technique and expe-

rience of the analysts resulted in "counts" which were even more regular and, therefore, presumably more accurate than before.

TABLE 5

Series C. Observed ratios between the counts from the 3 liters of inoculated milk. Theoretical ratio 1 : 2 : 4

ANALYST*	RATIO FOR PLATE "COUNTS"	ANALYST*	RATIO FOR GROUP "COUNTS"	RATIO FOR INDIVIDUAL "COUNTS"
BB	1:1.84:4.04	AA	1:2.00:3.79	1:1.94:3.80
BC	1:1.79:4.10	AI	1:2.02:4.32	1:2.04:4.43
CC	1:1.88:4.66	BB	1:1.80:3.96	1:1.89:4.03
CE	1:1.87:4.79	BH	1:1.59:5.98	1:1.70:6.67
EE	1:2.13:4.81	EE	1:1.94:4.02	1:1.90:3.92
EB	1:2.17:4.94	EF	1:2.15:4.85	1:2.15:4.81
FF	1:2.22:4.41	FF	1:1.81:3.28	1:1.90:3.16
FH	1:1.86:4.19	FE	1:2.13:3.61	1.2.15:3.62
HH	1:1.99:4.11	HH	1:1.92:3.88	1:1.99:4.33
HI	1:1.89:3.94	HB	1:1.98:4.17	1:1.90:4.08
II	1:2.02:4.60	II	1:1.94:4.25	1:1.94:4.11
IF	1:2.02:4.82	IA	1:2.01:4.35	1:2.06:4.41
Average...	1:1.97:4.43		1:1.95:4.11	1:1.97:4.12

* The first analyst named made the plates or slides. The second counted them.

TABLE 6

Series C. Summary of table 4

SAMPLE NUMBER	PLATE "COUNT"			GROUP "COUNT"			INDIVIDUAL "COUNT"		
	Final averages	Standard deviation	Coefficient variation	Final averages	Standard deviation	Coefficient variation	Final averages	Standard deviation	Coefficient variation
2–4	821,000	58,200	7.1	694,000	69,000	10.0	1,021,000	127,000	12.5
5–7	1,615,000	115,000	7.1	1,356,000	204,000	15.0	2,014,000	347,000	17.3
8–10	3,640,000	385,000	10.6	2,853,000	286,000	10.1	4,208,000	441,000	10.5
Average			8.3			11.7			13.4

TABLE 7

Series C. Maximum and minimum plate and microscopic "counts"

SAMPLE NUMBER	PLATE "COUNTS"		GROUP "COUNTS"		INDIVIDUAL "COUNTS"		TOTAL NUMBER OF "COUNTS"
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
1	450	1,150	Less than 3,000	27,000	Less than 3,000	30,000	12
	493	1,010	Less than 3,000	9,000	Less than 3,000	15,000	
2–4	703,000	937,000	252,000	930,000	354,000	1,326,000	36
	713,000	900,000	327,000	927,000	468,000	1,302,000	
5–7	1,380,000	1,870,000	435,000	2,295,000	663,000	3,033,000	36
	1,420,000	1,840,000	531,000	1,728,000	786,000	2,724,000	
8–10	2,950,000	4,600,000	1,632,000	3,900,000	2,463,000	5,268,000	36
	3,130,000	4,520,000	1,635,000	3,816,000	3,556,000	5,232,000	

b. Microscopic counts

However, the most striking change in the character of the "counts" obtained from series C, as contrasted with series B occurred in the microscopic "counts." As before, both group and individual "counts" were made.

Slides were prepared in duplicate from each sample, and all were recounted by a second analyst. As the slides were exchanged between the laboratories, they were in each case recounted by an analyst from the laboratory different from that in which the slides were made. All analysts standardized their microscopes, and used a special ocular micrometer as already described, so that each examined the central portion of the field only, and each examined 1/600,000 cc. of dried milk per field. A complete record was kept of the size of all groups seen.

The effect of the greater care taken in making the "counts," and in the increased skill gained through the experience of the previous series is seen in table 4, where the average "counts" are summarized. Even a cursory examination will show that these results harmonize much more closely than before, and that variations in count from the same samples as great as 100 per cent are practically absent. This is marked improvement over the results from series B as given in table 1.

Moreover, a study of the detailed records indicates that the less experienced men returned "counts" which bear evidence on their face of a greater accuracy than those returned by the same men from series B. None of the men, for example, found more than an occasional bacterium or pair of bacteria in the high grade fresh milk used in sample 1, whereas, in the previous series, the inexperienced men did not scrutinize the objects which they found in sample 1 closely enough, and evidently recorded and counted objects as bacteria which were not bacteria.

On the other hand, in the samples of inoculated milk where the bacteria were numerous, the less experienced men still showed a tendency to report fewer bacteria than did the experienced men who examined the identical slides. This effect is particularly noticeable in the "counts" made by the analyst (H) who had had the least experience in microscopic counting. This effect is chargeable to inexperience and not to carelessness as is shown by the fact that the plate "counts" returned by the men who made the irregular microscopic "counts" are as regular, and presumably as accurate, as any that were made.

An examination of the ratios, as given in table 5 shows that all of the analysts (with the single exception of analyst H) obtained ratios which agree well with the excepted ratio of 1:2:4. A marked improvement was shown in these over the similar ratios from series B. The final average ratios obtained were 1:1.95:4.11 for the group "counts" and 1:1.97:4.12 for the individual "counts." These are almost identical with the expected ratios, and are even more nearly perfect than the similar ratios from the plate "counts."

In order that the variations in "counts" may be studied more readily, the maximum and minimum "counts" returned by all of the analysts are given in table 7.

The greater regularity of these microscopic "counts" as contrasted with those of series B is brought out by a comparison of the coefficients of variability as recorded for series C on table 6 and for series B in table 3. All of the coefficients of variability are less for series C than those for series B, and in several instances indicate less variation of the microscopic "counts" than was

observed in the plate "counts" of series B. The average coefficient of variability for the group "counts" of series C is 11.7, while that for the individual "counts" is 13.4. These averages are decidedly better than the similar averages of 24.1 and 28.3 for series B. This improvement is undoubtedly to be charged to the greater skill of the analysts gained through their experience in the work of the previous series, and to the improved technique.

c. Comparison between the plate and microscopic counts

When the "counts" of series C as made by the two different methods are compared, the unique character of the results at once becomes evident. As already indicated, they meet the checks and counterchecks made upon their accuracy thus far discussed even more perfectly than those of series B.

They likewise very perfectly meet the test of comparison with each other, which is very severe in this case as the average number of individuals per group (1.45) was even less than before. However, in spite of this, every one of the final average plate "counts" as recorded in table 4 is intermediate in size between the group and individual "counts." Even the average "counts" as returned by the individual men showed only three of the group "counts" out of a possible 54 that were larger than the corresponding plate "counts," while only ten of the individual "counts" out of a possible 54 were smaller than the corresponding plate "counts" from the same samples. Moreover, eight of these were made by the one analyst (H) who, as already explained, evidently returned microscopic counts which were lower than they should have been.

Thus it becomes practically a certainty that there cannot be a large error in the final average figures, and that they actually show, in the one case, the number of groups originally present in the milk; in the second case, the number of centers of growth which existed after the partial distintegration of the clumps in the dilution waters; and, in the third case, the actual number of individual bacteria present.

Computation, based on the final average figures given in table 4, shows that, as in series B, the clumps were only partially broken apart in making the agar plates. Thus, whereas the average group of organisms in samples 2, 3, and 4 contained 1.47 individuals in the milk itself, the groups in the final dilutions used in making the plates contained 1.24 individuals. Likewise, whereas the original groups in the milk in samples 5, 6, and 7 contained 1.48 individuals, the groups in the final dilution water contained 1.25 individuals. In the third group of samples (8, 9, and 10), the average group in the milk itself contained 1.49 individuals, whereas the average group in the final dilution waters was reduced to 1.16 individuals. Thus, even under these very favorable conditions, the agar plate "counts" only approximate the number of bacteria present.

SERIES A. MISCELLANEOUS SAMPLES OF MILK

As this series of analyses has already been discussed in this journal (6) only brief reference will be made to it here. A summary of the results secured are given in table 8; while the detailed records appear in the bulletin by Breed and Stocking (10). From the detailed records it is possible to determine the causes of marked discrepancies in the plate and microscopic counts with a high degree of probability.

Sometimes the microscopic examination revealed the presence of masses of bacteria of such compact nature that they undoubtedly failed to break up completely. In other cases, the plates themselves gave evidence that some of the living bacteria had failed to develop into colonies. In general, however, it was the occurrence of the bacteria in groups which apparently caused the plate "counts" to be less than the probable number of bacteria present.

The average plate "counts" were larger than the group "counts" in 13 of the 21 samples (see table 8). In no case did the plate "count" exceed the individual "count" made from the same sample. Thus the plate "counts" ordinarily were intermediate in size between the two microscopic "counts," as was to be expected from theoretical considerations.

TABLE 8

Series A. Final summary of plate and microscopic "counts"

SAMPLE NUMBER	PLATE "COUNT"	GROUP "COUNT"	INDIVIDUAL "COUNT"	AVERAGE SIZE GROUP	NOTES
1	10,600	18,700	103,000	5.5	Herd milk
2	10,400	19,600	92,000	4.7	Herd milk
3–5	6,000	5,000	15,000	3.0	Herd milk
4	45,000	41,000	145,000	3.5	Herd milk
6	78,000	46,000	185,000	4.0	Market milk
7	72,000	47,000	202,000	4.3	Market milk
8	30,000	51,000	189,000	3.7	Market milk
9	98,000	80,000	383,000	4.8	Market milk
10	285,000	94,000	721,000	7.7	Market milk
11	52,000,000	93,000,000	99,000,000	1.06	Inoculated with long-rod, lactic acid organism
12	48,000,000	49,000,000	67,000,000	1.4	Inoculated with the colon bacillus
13	135,000,000	37,000,000	163,000,000	4.4	Fresh milk incubated at 37°C. for 24 hours
14	9,900,000	5,900,000	150,000,000	25.4	Market milk known to contain streptococci
15	2,000,000,000	1,500,000,000	2,700,000,000	1.8	Milk nearly curdled, containing *Bact. lactis acidi*
16	121,000	60,000	387,000	6.4	Market milk
17	23,000	13,000	234,000	18.0	Market milk found to contain streptococci
18	1,900	12,000	34,000	2.7	Market milk
19	12,000,000	8,400,000	28,000,000	3.3	Market milk
20	5,600,000	5,200,000	26,000,000	5.0	Market milk
21	390	3,000	4,000	1.3	Milk from single cow
22	623	5,500	7,250	1.3	Milk from single cow

DISCUSSION AND CONCLUSIONS

The results secured indicate that skilled analysts, using proper technique, ordinarily obtain reasonably accurate estimates of the number of living bacteria in cubic centimeter samples of milk by the plate method *provided* the milk contains isolated organisms of a type capable of growth on agar under the conditions maintained.

They likewise indicate that estimates of the numbers of living bacteria and of the groups of bacteria can be made with equal accuracy by direct microscopic examination *provided* there are neither large clumps of bacteria nor dead bacteria present.

These statements are based on the fact that, under the favorable conditions maintained in series B and C, six analysts obtained "counts" or "estimates" of the number of bacteria from duplicate samples of milk by the use of the agar plate technique and by direct microscopic examination which showed good agreement among themselves, which had a coefficient of variability under 15, and which met all other checks upon their accuracy in such a way as to establish their true accuracy with reasonable certainty.

Because of the presence of clumps of bacteria, the agar plate "counts" were in each case less than the true number of individual bacteria present.

It being self evident that these accurate estimates were obtained under conditions much more favorable than those ordinarily present, the question naturally arises whether the inaccuracies in "counts" under ordinary conditions are sufficient to destroy the value of bacterial determinations. This question cannot be answered with entire satisfaction even with the data gathered in this and in previous investigations; but certain facts are evident as the result of the work done on the series of miscellaneous samples.

There has been a general feeling in the past that the errors in plate "counts," which were of the greatest significance were those introduced through the failure to prepare media of the correct reaction or composition, through the failure to use proper incubation temperatures, or through spreading colonies, overcrowding of colonies, and the like. Much attention has been given to the standardization of technique in order to reduce the known difficulties to a minimum.

Nevertheless, it does not seem probable that the errors caused by these things are so generally present or so irregular as those caused by the occurrence of the bacteria in groups of different average sizes. As it is clearly evident from series A, it is not at

all uncommon to find that one milk contains bacteria occurring in groups averaging two individuals per group, while another contains bacteria occurring in groups averaging four, six or even more individuals. Large errors in count caused in this way must be the rule rather than the exception. The latter errors could be eliminated if some method could be devised whereby the groups could be broken into their component individuals before the plates were prepared, and recent discussions of the plating technique emphasize the necessity of shaking the samples in a standardized way. Bacteria have so little weight, however, in proportion to their surface that hand shaking can have but little effect upon the compact groups.

The most disquieting feature of the errors introduced by the clumping of the bacteria is the fact that they may be highly variable, and yet pass undetected. It is reassuring to secure a series of plate "counts" from duplicate samples of milk which are in close agreement; and especially so if the analyses are made in different laboratories. However, the feeling that the "counts" are accurate passes away at once as soon as the microscopic studies are made, and it is found probable that some of the "counts" are affected by a 100 per cent error, others by a 200 per cent error, and still others by a 1000 per cent error, none of which are indicated in any way in the plate "counts."

It is natural to feel that a milk sample giving a plate "count" of 40,000 per cubic centimeter actually contains fewer bacteria than a milk sample giving a plate "count" of 50,000 per cubic centimeter. Yet comparative studies indicate that the chances are at least as great as one out of three that milk giving a plate "count" of 50,000 actually contains fewer bacteria than milk giving a plate "count" of 40,000. The chance that the lower "count" signifies larger numbers of bacteria becomes less and less as the margin between the "counts" becomes greater. If one can judge from the records already available giving the average size of the groups of bacteria in miscellaneous samples of milk, a margin of one to five makes it practically certain that the sample giving the larger "count" is actually from the milk containing the larger number of bacteria per cubic centimeter;

but even this margin does not appear to be sufficient to cover the errors introduced through the clumping of streptococci.

In contrast to these errors in plate "counts" caused by the difficulties involved in devising a perfect technique, the greatest difficulties met with in making accurate microscopic "counts" are those which involve the skill and patience of the analyst. Thus, in the present work, it was not until the last series of analyses were made that the men without previous experience realized these limitations of the microscopic technique, and not until then did the work of all the men begin to have precision and accuracy. In this series (C) the coefficient of variability of the microscopic "counts" was reduced to a figure comparable with that obtained from the plate "counts," i.e., less than 15.

Because the "counts" were made by two methods, it was ordinarily possible to detect conditions unfavorable to accurate work. Thus the microscope always showed what the conditions really were in regard to the size of the groups and the types of bacteria present, while the agar plates showed the number of centers of growth capable of development under the conditions maintained. Gross discrepancies in the "counts," not explainable through the presence of clumps, suggested the presence of dead bacteria, or of living bacteria not capable of growth on the plates. The fact that interpretations of this sort were possible shows that it would be an excellent practice for analysts to make "counts" of both sorts wherever especially accurate results are desired. Under such conditions care should be taken to make the "counts" by more precise methods than those used in ordinary routine work.

SUMMARY

1. Three series of bacterial counts from samples of fresh, unpasteurized milk have been completed. Six or seven analysts participated in each, working in two groups in laboratories located within fifty miles of each other.

2. In all cases, counts were made both by the agar plate method and by direct microscopic examination, thus permitting

a check upon the accuracy of the counts not possible where only one method of counting is used.

3. In two series (B and C), the samples analyzed were carefully prepared so as to present the most favorable conditions possible for accurate counting and to allow checks to be made upon the accuracy of the results. This was accomplished by inoculating three lots of freshly drawn milk known to contain very few bacteria, with a skim milk culture of the colon organism. The amount of inoculum used was such that the final counts were expected to show the ratio 1:2:4. The colon organism was chosen because it grows well under normal conditions, and exists in milk largely as isolated individuals.

4. Under the above conditions, the results met all of the checks upon their accuracy so perfectly that there can be little doubt but that they actually were fairly accurate counts of the number of individual bacteria present.

5. The results obtained in the final series (C) were so uniform that the coefficient of variability was reduced to less than 15 in all cases. Under the conditions present, the variability of the microscopic counts was slightly greater than that of the agar plate counts.

6. In the counts made from samples containing a miscellaneous flora (series A), wide variations were found between the plate and microscopic counts. The primary cause of these variations appeared to be the existence of clumps of bacteria which were not separated into their component individuals in preparing the agar plates. The uniformity of the agar plate counts was generally good, indicating that the technique used was satisfactory. The greater lack of uniformity in the microscopic counts was in part due to the inexperience of some of the analysts, several of whom had never before attempted to make accurate counts by microscopical methods.

7. The average number of individuals in the clumps of bacteria present commonly varied between two and six; but at times (when streptococci were present) greatly exceeded these numbers. As the data indicate that the clumps are only very poorly broken apart in the processes ordinarily used in preparing dilu-

tion waters, the plate counts did not represent the full number of bacteria present.

8. The chief limitations upon the accuracy of the microscopic counts appear to be those involving the skill of the analyst making the microscopic observations, and the patience necessary in order to examine a sufficiently large quantity of milk to give an accurate average. Given unlimited time, and numerous duplicate preparations from a sample of milk, a skilled microscopist can secure reasonably accurate counts of the number of individual bacteria present in any ordinary sample of milk. Yet the laboriousness of this proceeding limits its usefulness, and makes it impossible to actually count the bacteria in examining large numbers of samples.

9. Fortunately neither the inaccuracy of the plate counts caused by the clumping nor the limitations of the microscopic technique just noted, appear to be so great as to prevent the use of either technique where the purpose is to grade miscellaneous samples of unpasteurized milk into two or three grades. However, the information at present available indicates that attempts to use simplified methods for analysis for the purpose of making finer distinctions in quality introduces gross errors. When a finer classification is desired (as is now the case in many grade A plants) the use of the so-called simplified routine control methods should not be regarded as satisfactory.* The present ituation suggests the desirability of the State exercising controls over bacteriological methods for analyses, whenever the results are to be used as a basis for payment in order to insure the use of more accurate methods of analysis just as it now does in the case of the Babcock test for determining the percentage of butter fat.

REFERENCES

(1) Conn, H. W.: Standards for determining the purity of milk. Public Health Repts. of U. S. Pub. Health Ser., xxx, 2349–2395, 1915.

(2) Hatfield, Hazel M.: A comparative study of milk plates by four New York laboratories. A preliminary report. Amer. Jour. Pub. Health, viii, 913–915, 1918.

(3) Kilbourne, C. H.: Varying bacteriological results obtained by different laboratories. Creamery and Milk Plant Monthly, vii, nos. 10 and 11, 1918.

(4) BREW, J. D.: A comparison of the microscopical method and the plate method of counting bacteria in milk. N. Y. Agr. Exp. Sta., Bul. 373, 1914.

(5) BREW, J. D., AND DOTTERRER, W. D.: The number of bacteria in milk. N. Y. Agr. Exp. Sta., Bul. 439, 1917.

(6) BREED, R. S., AND STOCKING, W. A.: A preliminary report on a series of coöperative bacterial analyses of milk. Jour. Dairy Sci., i, 19–35, 1917.

(7) BREED, R. S., AND BREW, J. D.: Counting bacteria by means of the microscope. N. Y. Agr. Exp. Sta., Tech. Bul. 49, 1916.

(8) Report Committee on Standard Methods Milk Analysis. Amer. Jour. Pub. Hyg., xx (N.S. 6), 315–345, 1910 (First edition). Amer. Jour. Pub. Health, vi, 1315–1325, 1916 (Second edition).

(9) FROST, W. D.: Rapid method of counting bacteria in milk. Sci., xlii, 255–256, 1915.

(10) BREED, R. S., AND STOCKING, W. A.: The accuracy of bacterial counts from milk samples. N. Y. Agr. Exp. Sta., Tech. Bul. 75, 1920.

OPEN FORUM

Should We Have a New Milk Score Card?

Score cards for butter and cheese have been in use for many years. Score cards for dairies were formulated almost simultaneously, in 1904, by Woodward at Washington and Pearson at Cornell University. These score cards were but the first of many. The flood gates of pent up enthusiasm for scoring opened, with the result that there were soon score cards for everything connected with the farm, including score cards for judging husbands and wives.

Recognizing the desirability of a uniform score card for dairy farms, the Official Dairy Instructors Association, or as it is now called, the American Dairy Science Association, appointed a Committee on Dairy Score Cards (1). This committee formulated, and in 1908 the Association adopted, the so-called Official Dairy Score Card, which has been widely used in the inspection of dairy farms. This score card is "Official" in the sense that it has been adopted by the Official Dairy Instructors Association and in many cities it is also "Official" in the sense that it has been legally made a part of the local health regulations.

A majority of the score cards of various kinds which have been proposed have been forgotten and the making of score cards has gone rather out of fashion. The score card for milk is an exception to this general statement. The milk score card was originally developed by the Federal Dairy Division for scoring bottles of milk in competition at various milk shows and it has undoubtedly served a useful purpose. This card is in a sense the property of the Federal Dairy Division and no one should question their right to continue it or change it as they see fit.

In 1919 (2), recognizing the increasing public interest in food value as an element of milk quality, the members of the Dairy Division decided to change their card by adding 10 points to those allotted to fat and solids not fat. To keep the total on the card at 100 points they subtracted these 10 points from the 25 formerly allotted to flavor and odor. This adaptation of the score card to changing conditions is commendable.

In order that they may be more readily discussed, both of the present milk score cards are reproduced in table 1.

One of these milk score cards has been in use for a number of years at the National Dairy Show. As a part of the educational work at the Dairy Show it has been the custom to stage a scoring contest in which teams representing Agricultural Colleges compete for medals and other prizes. The milk score card has been used in scoring the milk in this contest.

After the appearance of the revised milk score card, in August, 1919, a misunderstanding arose among the coaches of these teams as to which score card was to be used in the Dairy Show Contest. As a result some

TABLE 1

Present score cards for milk

	PERFECT	
Bacteria	35	35
Flavor and odor	25	15
Visible dirt	10	10
Fat	10	15
Solids not fat	10	15
Acidity	5	5
Bottle and cap	5	5
	100	100

of the teams were at a disadvantage because they were trained in the use of one card and were compelled to compete in the use of the other.

Feeling the need of an official score card for milk, a committee on this subject was appointed in 1919 by the American Dairy Science Association. This committee recommended that the earlier form of Federal Milk Score Card be used in competitions for the year 1920 and at the recent annual meeting of the Association again recommended that the use of this card be continued for one year, pending their presentation in 1921 of a card for adoption by the Association. This leaves the matter of a milk score card before the Association without any prejudice as to the form of card to be later adopted, but makes it desirable that the members of the Association place their suggestions on this subject at the service of the committee.

The form of score card should depend primarily on the object to be attained through its use. The primary object of the card is to offer

a just basis for deciding which entry among those competing is the best milk. Back of the competition lies the desire on the part of the producer or dealer to gain favorable recognition for his dairy. The public quite uniformly consider that the winning of a prize in such a competition is evidence that the milk furnished by that dairy is of special excellence.

In addition to this primary aim of the card there is the possibility of developing a card which will accurately measure the real desirability of the milk. The real object of all attempts at safe-guarding public milk supplies is to find and encourage the production of high grade milk. If the quality of the milk as delivered to the consumer can be accurately measured by a score card, milk supervision can be largely reduced to establishing milk grades and to determining that the milk as delivered is sold in strict accordance with these grades.

The question of what are the elements, which taken together establish the quality of milk, was decided by the American Dairy Science Association when, in 1918, it adopted the report of its Committee on Milk Quality (3). This report stated that the quality of milk depends upon (1) food value; (2) healthfulness; (3) cleanliness; and (4) keeping quality. These qualities exactly parallel the oft repeated inquiries of the consumer: Is the milk rich? Is it safe? Is it clean? Is it sweet? If the score on a milk score card is to accurately measure the quality of the milk, the card must give due consideration to all of the elements of milk quality. In order to make plain the extent to which the present score cards do give such consideration, the score cards as shown in table 1 may be rearranged under the above four headings of milk quality. The cards so rearranged are shown in table 2.

The above table brings out the important and surprising fact that the present score cards entirely disregard the question of the safety of the milk. Under such conditions the gold medal may go to a highly dangerous milk supply. This entails not only an injustice to the competitors but also the added danger that the unsuspecting public, noting the receipt of this prize, may be stimulated to consume this dangerous milk. It should be noted that the public, by using their powers of observation, can reasonably assure themselves regarding the richness, cleanliness and sweetness of a milk supply but that they are practically helpless in attempting in this way to measure the safety of their milk supply. For this reason the Health Officials find their main problem the protection of the safety of the milk. On this account it is all the more surprising that the present milk score cards not only disregard

entirely the question of safety but actually mislead the consumer at the very point where he most needs help.

This entire omission of what many would consider the most important point in milk quality is not particularly evident from casual inspection of these cards because these cards blend together their results into a single score. Those familiar with milk will recognize that the presence

TABLE 2

Present score cards rearranged

	PERFECT	
Food value		
Fat	10	15
Solids not fat	10	15
Healthfulness		
Tuberculin test or pasteurization	0	0
Cleanliness		
Visible dirt	10	10
Bottle and cap	5	5
Keeping quality		
Bacteria	35	35
Flavor and odor	25	15
Acidity	5	5
	100	100

TABLE 3

Suggested score card for milk

DIVISION		PERFECT
I	Food value	
	Fat	15
	Solids not fat	10
II	Healthfulness	25
III	Cleanliness	
	Visible dirt	25
IV	Keeping Quality	
	Flavor and odor	15
	Bacteria	5
	Acidity	5
		100

When the score of each division is 20 or above the milk is *good.*
When the score of each division is 23 or above the milk is *excellent.*

of an unusual amount of one element of quality such as food value will not satisfactorily off-set either the presence of large amounts of dirt or the fact that the milk is sour. If any of the four elements of quality are distinctly deficient the milk must be considered as poor. On the other hand, if the milk scores high with regard to all four elements of quality the milk is good.

Accordingly it would seem that the two outstanding deficiencies of the present milk score cards were (1) the lack of proper attention to the safety of the milk; and (2) the failure to keep separate the score of each of the four distinct elements of milk quality.

It is rather easy to correct both of these weaknesses and an illustration of one method of doing this is given in table 3.

It should be clearly understood that the score card shown in table 3 is not offered as a perfect score card. It is presented in order to illustrate how, with the minimum change of the present card, the criticisms which have been raised against the present cards may be met.

The principle of recognizing divisions of the card, which divisions are considered separately in determining the quality of the milk, is neither new nor untried. It was one of the best features in the so-called Cornell Dairy Farm Score Card, devised by President R. A. Pearson and used successfully for some years by a number of municipalities (4).

The fundamental difficulty with the various past attempts at grading milk supplies has been the knowledge that the grades thus established were largely artificial and did not correspond with the real quality of the milk. If a milk score card can be agreed upon in which all the elements of milk quality enter proportionately, its application to the grading of milk is a short step and one which can be easily taken.

REFERENCES

(1) Lane, C. B., and Whitaker, G. M.: The score card system of dairy inspection. U. S. D. A., B. A. I. Circ. 139, 1909.

(2) Kelly, E., and Taylor, G. B.: Milk and cream contests. U. S. D. A., Circ. 53, 1919.

(3) Harding, H. A., Breed, R. S., Stocking, W. A., Jr., and Hasting, E. G.: What is meant by "quality" in milk. Jour. Dairy Sci., i, 199–217, 1917.

(4) Harding, H. A.: Publicity and payment on quality as factors in improving a city milk supply. N. Y. Agr. Exp. Sta. Bul. 337. 1911. An. Rept. 30 (1911): 31–66. 1912.

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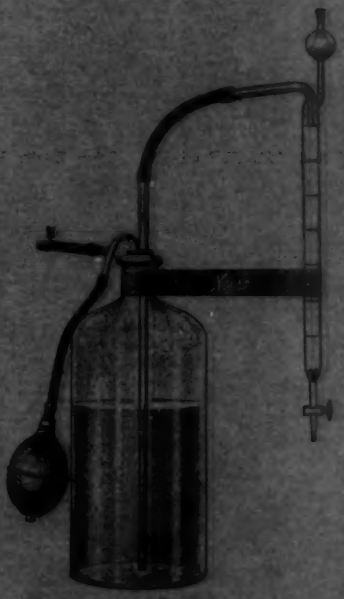

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